PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

AB

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61K 39/395, 39/40, G01N 33/53, 33/567

A1

(11) International Publication Number: **WO 97/48418**

(43) International Publication Date: 24 December 1997 (24.12.97)

(21) International Application Number: PCT/US97/10769

(22) International Filing Date: 20 June 1997 (20.06.97)

(30) Priority Data:
08/667,769 21 June 1996 (21.06.96) US

(60) Parent Application or Grant
(63) Related by Continuation
US 08/667,769 (CON)
Filed on 21 June 1996 (21.06.96)

(71) Applicant *(for all designated States except US)*: SMITHKLINE BEECHAM CORPORATION [US/US]; One Franklin Plaza, Philadelphia, PA 19103 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: APPELBAUM, Edward, Robert [US/US]; 830 Village Circle, Blue Bell, PA 19422 (US). COOK, Richard, Murray [GB/US]; 1006 Country Way, Chester Springs, PA 19425 (US).

(74) Agents: EAGLE, Alissa, M. et al.; SmithKline Beecham Corporation, Corporate Intellectual Property, UW2220, 709 Swedeland Road, P.O. Box 1539, King of Prussia, PA 19406-0939 (US).

(81) Designated States: AL, AM, AU, BB, BG, BR, CA, CN, CZ, EE, GE, GH, HU, IL, IS, JP, KG, KP, KR, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, US, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: IMPROVED METHOD FOR TREATMENT AND DIAGNOSIS OF IL-5 MEDIATED DISORDERS

(57) Abstract

The present invention relates to treatment and diagnosis of conditions mediated by IL-5 and excess eosinophil production, and more specifically to mAbs and other altered antibodies such as Fabs, chimeric, human and humanized antibodies that do not block binding of human IL-5 to the α-chain of the human IL-5 receptor.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

IMPROVED METHOD FOR TREATMENT AND DIAGNOSIS OF IL-5 MEDIATED DISORDERS

Cross Reference to Related Applications

This application is a continuation-in-part of PCT/US95/17082 filed December 22, 1995, which is a continuation-in-part of U.S. Serial Nos. 08/470,110 and 08/467,420, both filed June 6, 1995, which are continuation-in-parts of U.S. Serial No. 08/363,131 filed December 23, 1994.

Field of the Invention

The present invention relates generally to treatment and diagnosis of conditions mediated by IL-5 and excess eosinophil production, and more specifically to mAbs and other altered antibodies such as Fabs, chimeric, human and humanized antibodies.

Background of the Invention

Eosinophils have been implicated in the pathogenesis of a wide variety of inflammatory disease states including allergic disorders associated with hypersensitivity reactions in the lung tissue (Butterfield et al., In: Immunopharmacology of Eosinophils, H. Smith and R. Cook, Eds., p.151-192, Academic Press, London (1993)). A notable example is asthma, a disease characterized by reversible obstruction of the airways leading to non-specific bronchial hyperresponsiveness. This in turn is dependent upon the generation of a chronic inflammatory reaction at the level of the bronchial mucosa and a characteristic infiltration by macrophages, lymphocytes and eosinophils. The eosinophil appears to play a central role in initiating the mucosal damage typical of the disease (Corrigan et al., Immunol. Today, 13:501-507 (1992)). Increased numbers of activated eosinophils have been reported in the circulation, bronchial secretions and lung parenchyma of patients with chronic asthma, and the severity of the disease, as measured by a variety of lung function tests, correlates with blood eosinophil numbers (Griffen et al., J. Aller. Clin. Immunol., 67:548-557 (1991)). Increased numbers of eosinophils, often in the process of degranulation, have also been recovered in bronchoalveolar lavage (BAL) fluids of patients undergoing late asthmatic reactions, and reducing eosinophil numbers, usually as a consequence of steroid therapy, is associated with improvements in clinical symptoms (Bousquet et al., N. Eng. J. Med., 323:1033-1039 (1990)).

Interleukin 5 (IL-5) is a homodimeric glycoprotein produced predominantly by activated CD4+ T lymphocytes. In man, IL-5 is largely responsible for controlling the growth and differentiation of eosinophils. Elevated levels of IL-5 are detected in the bronchoalveolar lavage washings of asthmatics (Motojima et al., Allergy, 48:98

(1993)). Mice which are transgenic for IL-5 show a marked eosinophilia in peripheral blood and tissues in the absence of antigenic stimulation (Dent et al., J. Exp. Med., 172:1425 (1990)) and anti-murine IL-5 monoclonal antibodies have been shown to have an effect in reducing eosinophilia in the blood and tissues of mice (Hitoshi et al., Int. Immunol., 3:135 (1991)) as well as the eosinophilia associated with parasite infection and allergen challenge in experimental animals (Coffman et al., Science, 245:308-310 (1989), Sher et al., Proc. Natl. Acad. Sci., 83:61-65 (1990), Chand et al., Eur. J. Pharmacol., 211:121-123 (1992)).

Although corticosteroids are extremely effective in suppressing eosinophil numbers and other inflammatory components of asthma, there are concerns about their side effects in both severe asthmatics and more recently in mild to moderate asthmatics. The only other major anti-inflammatory drug therapies - cromoglycates (cromolyn sodium and nedocromil) - are considerably less effective than corticosteroids and their precise mechanism of action remains unknown.

More recent developments have focused on new inhaled steroids, longer acting bronchodilators and agents acting on novel biochemical or pharmacological targets (e.g., potassium channel activators, leukotriene antagonists, 5-lipoxygenase (5-LO) inhibitors etc.). An ideal drug would be one that combines the efficacy of steroids with the safety associated with cromolyn sodium, yet has increased selectivity and more rapid onset of action. Neutralizing IL-5 antibodies may potentially be useful in relieving eosinophila-related symptoms in man.

Hence there is a need in the art for a high affinity IL-5 antagonist, such as a neutralizing monoclonal antibody to human interleukin 5, which would reduce eosinophil differentiation and proliferation (i.e., accumulation of eosinophils) and thus eosinophil inflammation.

Summary of the Invention

In a first aspect, the present invention provides an improved method for treating conditions associated with excess eosinophil production wherein the improvement comprises the step of administering a neutralizing monoclonal antibody for human IL-5, which does not block binding of human IL-5 to the human IL-5 receptor a-chain. Exemplary of such monoclonal antibodies is rat monoclonal antibody 4A6.

In yet another aspect of the invention is a method to assess the presence or absence of a human IL-5 soluble receptor α-chain/human IL-5 complex in a human which comprises obtaining a sample of biological fluid from a patient and allowing a monoclonal antibody for human IL-5 which does not block binding of human IL-5 to the α-chain of the human IL-5 receptor to come in contact with such sample under

conditions such that a human IL-5 soluble receptor α-chain/human IL-5/monoclonal antibody complex can form and detecting the presence or absence of said human IL-5 soluble receptor α-chain/human IL-5/ monoclonal antibody complex. This method can be used to diagnose conditions associated with excess eosinophil production in a human and also to track progress and treatment of such disorders.

In a further aspect, the present invention provides a method of screening compounds to identify those compounds which antagonize binding of IL-5, IL-5/IL-5 receptor α-chain complex, or IL-5/IL-5 receptor α-chain/mAb complex to a human IL-5 receptor β-chain which comprises contacting the human IL-5 receptor β-chain with a plurality of candidate compounds under conditions to permit binding to the IL-5 receptor β-chain and identifying those candidate compounds that antagonize binding of said IL-5, IL-5/IL-5 receptor α-chain complex, or IL-5/IL-5 receptor α-chain/mAb complex to the IL-5 receptor β-chain.

Other aspects and advantages of the present invention are described further in the detailed description and the preferred embodiments thereof.

Brief Description of the Drawings

FIG. 1 [SEQ ID NOS: 1 and 15] illustrates the heavy chain variable region for the murine antibody 2B6, and the murine/human 2B6 chimeric antibody. The boxed areas indicate the CDRs.

FIG. 2 [SEQ ID NOs: 2 and 16] illustrates the light chain variable region for the murine antibody 2B6, and the murine/human 2B6 chimeric antibody. The boxed areas indicate the CDRs.

FIG. 3 [SEQ ID NO:3] illustrates the heavy chain variable region for the murine antibody 2F2. The boxed areas indicate the CDRs.

FIG. 4 [SEQ ID NO:4] illustrates the light chain variable region for the murine antibody 2F2. The boxed areas indicate the CDRs.

FIG. 5 [SEQ ID NO:5] illustrates the heavy chain variable region for the murine antibody 2E3. The boxed areas indicate the CDRs.

FIG. 6 [SEQ ID NO:6] illustrates the light chain variable region for the murine antibody 2E3. The boxed areas indicate the CDRs.

FIG. 7 [SEQ ID NOs:7-14] illustrates the heavy and light chain CDRs from murine antibodies 2B6, 2F2 and 2E3.

FIG. 8 [SEQ ID NOs: 18, 19] illustrates the heavy chain variable region for the humanized antibody 2B6. The boxed areas indicate the CDRs.

FIG. 9 [SEQ ID NOs: 20, 21] illustrates the light chain variable region for the humanized antibody 2B6. The boxed areas indicate the CDRs.

FIG. 10 is a schematic drawing of plasmid pCDIL5HZHC1.0 employed to express a humanized heavy chain gene in mammalian cells. The plasmid contains a beta lactamase gene (BETA LAC), an SV-40 origin of replication (SV40), a cytomegalovirus promoter sequence (CMV), a signal sequence, the humanized heavy chain, a poly A signal from bovine growth hormone (BGH), a betaglobin promoter (beta glopro), a dihydrofolate reductase gene (DHFR), and another BGH sequence poly A signal in a pUC19 background.

FIG. 11 is a schematic drawing of plasmid pCNIL5HZLC1.0 employed to express a humanized light chain gene in mammalian cells.

FIG. 12 [SEQ ID NOs: 61, 62] illustrates the NewM heavy chain variable region for the humanized antibody 2B6. The boxed areas indicate the CDRs.

FIG. 13 [SEQ ID NOs: 69, 70] illustrates the REI light chain variable region for the humanized antibody 2B6. The boxed areas indicate the CDRs.

Detailed Description of the Invention

The present invention provides a variety of antibodies, altered antibodies and fragments thereof, which are characterized by human IL-5 binding specificity, neutralizing activity, and high affinity for human IL-5 as exemplified in murine monoclonal antibody 2B6 or rat antibody 4A6. The antibodies of the present invention were prepared by conventional hybridoma techniques, phage display combinatorial libraries, immunoglobulin chain shuffling, and humanization techniques to generate novel neutralizing antibodies. These products are useful in therapeutic and pharmaceutical compositions for treating IL-5-mediated disorders, e.g., asthma. These products are also useful in the diagnosis of IL-5-mediated conditions by measurement (e.g., enzyme linked immunosorbent assay (ELISA)) of endogenous IL-5 levels in humans or IL-5 released *ex vivo* from activated cells.

Preferably the antibodies of the invention bind to human IL-5, but do not block the interaction between human IL-5 and IL-5 receptor α-chain. That is, the preferred antibodies are non-competitive with the IL-5 receptor α-chain for human IL-5. The preferred antibodies of the invention also bind to an IL-5/IL-5 receptor α-chain complex. A naturally-occurring soluble form of the IL-5 receptor α-chain has been observed *in vitro* (see, e.g., Tavernier et al., Cell, 66:1175-1184 (1991)) but it was not known, prior to the this invention, whether a soluble form of the IL-5 receptor α-chain was produced *in vivo*. Applicants have identified a soluble form of the IL-5 receptor α-chain *in vivo*. Thus, an antibody that binds to complexed IL-5/IL-5 receptor α-chain would be a more effective therapeutic for it would bind "free" or uncomplexed IL-5 as well as complexed IL-5. In addition, the soluble form of the IL-5 receptor α-chain

may be a natural antagonist of human IL-5. Hence, an antibody that does not compete with the soluble receptor is a more desirable and effective antagonist of IL-5, and thus it is an improved therapeutic (relative to mAbs that do compete with the IL-5 receptor α-chain for binding to IL-5) for treating IL-5 mediated conditions such as excess eosinophil production.

I. Definitions.

"Altered antibody" refers to a protein encoded by an altered immunoglobulin coding region, which may be obtained by expression in a selected host cell. Such altered antibodies are engineered antibodies (e.g., chimeric or humanized antibodies) or antibody fragments lacking all or part of an immunoglobulin constant region, e.g., Fv, Fab, or $F(ab)_2$ and the like.

"Altered immunoglobulin coding region" refers to a nucleic acid sequence encoding altered antibody of the invention. When the altered antibody is a CDR-grafted or humanized antibody, the sequences that encode the complementarity determining regions (CDRs) from a non-human immunoglobulin are inserted into a first immunoglobulin partner comprising human variable framework sequences. Optionally, the first immunoglobulin partner is operatively linked to a second immunoglobulin partner.

"First immunoglobulin partner" refers to a nucleic acid sequence encoding a human framework or human immunoglobulin variable region in which the native (or naturally-occurring) CDR-encoding regions are replaced by the CDR-encoding regions of a donor antibody. The human variable region can be an immunoglobulin heavy chain, a light chain (or both chains), an analog or functional fragments thereof. Such CDR regions, located within the variable region of antibodies (immunoglobulins) can be determined by known methods in the art. For example Kabat et al. (Sequences of Proteins of Immunological Interest, 4th Ed., U.S. Department of Health and Human Services. National Institutes of Health (1987)) disclose rules for locating CDRs. In addition, computer programs are known which are useful for identifying CDR regions/structures.

"Neutralizing" refers to an antibody that inhibits IL-5 activity by preventing the binding of human IL-5 to its specific receptor or by inhibiting the signaling of IL-5 through its receptor, should binding occur. A mAb is neutralizing if it is 90% effective, preferably 95% effective and most preferably 100% effective in inhibiting IL-5 activity as measured in the B13 cell bioassay (IL-5 Neutralization assay, see Example 2C).

The term "high affinity" refers to an antibody having a binding affinity characterized by a K_d equal to or less than 3.5×10^{-11} M for human IL-5 as determined by optical biosensor anaylsis (see Example 2D).

By "binding specificity for human IL-5" is meant a high affinity for human, not murine, IL-5.

"Second immunoglobulin partner" refers to another nucleotide sequence encoding a protein or peptide to which the first immunoglobulin partner is fused in frame or by means of an optional conventional linker sequence (i.e., operatively linked). Preferably it is an immunoglobulin gene. The second immunoglobulin partner may include a nucleic acid sequence encoding the entire constant region for the same (i.e., homologous - the first and second altered antibodies are derived from the same source) or an additional (i.e., heterologous) antibody of interest. It may be an immunoglobulin heavy chain or light chain (or both chains as part of a single polypeptide). The second immunoglobulin partner is not limited to a particular immunoglobulin class or isotype. In addition, the second immunoglobulin partner may comprise part of an immunoglobulin constant region, such as found in a Fab, or $F(ab)_2$ (i.e., a discrete part of an appropriate human constant region or framework region). Such second immunoglobulin partner may also comprise a sequence encoding an integral membrane protein exposed on the outer surface of a host cell, e.g., as part of a phage display library, or a sequence encoding a protein for analytical or diagnostic detection, e.g., horseradish peroxidase, β-galactosidase, etc.

The terms Fv, Fc, Fd, Fab, or $F(ab)_2$ are used with their standard meanings (see, e.g., Harlow et al., Antibodies A Laboratory Manual, Cold Spring Harbor Laboratory, (1988)).

As used herein, an "engineered antibody" describes a type of altered antibody, i.e., a full-length synthetic antibody (e.g., a chimeric or humanized antibody as opposed to an antibody fragment) in which a portion of the light and/or heavy chain variable domains of a selected acceptor antibody are replaced by analogous parts from one or more donor antibodies which have specificity for the selected epitope. For example, such molecules may include antibodies characterized by a humanized heavy chain associated with an unmodified light chain (or chimeric light chain), or vice versa. Engineered antibodies may also be characterized by alteration of the nucleic acid sequences encoding the acceptor antibody light and/or heavy variable domain framework regions in order to retain donor antibody binding specificity. These antibodies can comprise replacement of one or more CDRs (preferably all) from the acceptor antibody with CDRs from a donor antibody described herein.

A "chimeric antibody" refers to a type of engineered antibody which contains naturally-occurring variable region (light chain and heavy chains) derived from a donor antibody in association with light and heavy chain constant regions derived from an acceptor antibody.

A "humanized antibody" refers to a type of engineered antibody having its CDRs derived from a non-human donor immunoglobulin, the remaining immunoglobulin-derived parts of the molecule being derived from one (or more) human immunoglobulin(s). In addition, framework support residues may be altered to preserve binding affinity (see, e.g., Queen et al., Proc. Natl Acad Sci USA, 86:10029-10032 (1989), Hodgson et al., Bio/Technology, 9:421 (1991)).

The term "donor antibody" refers to an antibody (monoclonal, or recombinant) which contributes the nucleic acid sequences of its variable regions, CDRs, or other functional fragments or analogs thereof to a first immunoglobulin partner, so as to provide the altered immunoglobulin coding region and resulting expressed altered antibody with the antigenic specificity and neutralizing activity characteristic of the donor antibody. One donor antibody suitable for use in this invention is a non-human neutralizing monoclonal antibody (i.e., murine) designated as 2B6. The antibody 2B6 is defined as a high affinity, human-IL-5 specific (i.e., does not recognize murine IL-5), neutralizing antibody of isotype IgG_1 having the variable light chain DNA and amino acid sequences of SEQ ID NOs: 2 and 16, respectively, and the variable heavy chain DNA and amino acid sequences of SEQ ID NOs: 1 and 15, respectively, on a suitable murine IgG constant region.

The term "acceptor antibody" refers to an antibody (monoclonal; or recombinant) heterologous to the donor antibody, which contributes all (or any portion, but preferably all) of the nucleic acid sequences encoding its heavy and/or light chain framework regions and/or its heavy and/or light chain constant regions to the first immunoglobulin partner. Preferably a human antibody is the acceptor antibody.

"CDRs" are defined as the complementarity determining region amino acid sequences of an antibody which are the hypervariable regions of immunoglobulin heavy and light chains. See, e.g., Kabat et al., Sequences of Proteins of Immunological Interest, 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987). There are three heavy chain and three light chain CDRs (or CDR regions) in the variable portion of an immunoglobulin. Thus, "CDRs" as used herein refers to all three heavy chain CDRs, or all three light chain CDRs (or both all heavy and all light chain CDRs, if appropriate).

CDRs provide the majority of contact residues for the binding of the antibody to the antigen or epitope. CDRs of interest in this invention are derived from donor antibody variable heavy and light chain sequences, and include analogs of the naturally occurring CDRs, which analogs also share or retain the same antigen binding specificity and/or neutralizing ability as the donor antibody from which they were derived.

By 'sharing the antigen binding specificity or neutralizing ability' is meant, for example, that although mAb 2B6 may be characterized by a certain level of antigen affinity, a CDR encoded by a nucleic acid sequence of 2B6 in an appropriate structural environment may have a lower, or higher affinity. It is expected that CDRs of 2B6 in such environments will nevertheless recognize the same epitope(s) as 2B6. Exemplary heavy chain CDRs of 2B6 include SEQ ID NO: 7; SEQ ID NO: 8; SEQ ID NO: 9; and exemplary light chain CDRs of 2B6 include SEQ ID NO: 10; SEQ ID NO: 11; and SEQ ID NO: 12.

A "functional fragment" is a partial heavy or light chain variable sequence (e.g., minor deletions at the amino or carboxy terminus of the immunoglobulin variable region) which retains the same antigen binding specificity and/or neutralizing ability as the antibody from which the fragment was derived.

An "analog" is an amino acid sequence modified by at least one amino acid, wherein said modification can be chemical or a substitution or a rearrangement of a few amino acids (i.e., no more than 10), which modification permits the amino acid sequence to retain the biological characteristics, e.g., antigen specificity and high affinity, of the unmodified sequence. For example, (silent) mutations can be constructed, via substitutions, when certain endonuclease restriction sites are created within or surrounding CDR-encoding regions.

Analogs may also arise as allelic variations. An "allelic variation or modification" is an alteration in the nucleic acid sequence encoding the amino acid or peptide sequences of the invention. Such variations or modifications may be due to degeneracy in the genetic code or may be deliberately engineered to provide desired characteristics. These variations or modifications may or may not result in alterations in any encoded amino acid sequence.

The term "effector agents" refers to non-protein carrier molecules to which the altered antibodies, and/or natural or synthetic light or heavy chains of the donor antibody or other fragments of the donor antibody may be associated by conventional means. Such non-protein carriers can include conventional carriers used in the diagnostic field, e.g., polystyrene or other plastic beads, polysaccharides, e.g., as used in the BIAcore [Pharmacia] system, or other non-protein substances useful in the

medical field and safe for administration to humans and animals. Other effector agents may include a macrocycle, for chelating a heavy metal atom, or radioisotopes. Such effector agents may also be useful to increase the half-life of the altered antibodies, e.g., polyethylene glycol..

"Conditions associated with excess eosinophil production" refer to allergic and/or atopic responses, or to responses associated with eosinophilia, such as but not limited to, allergic rhinitis and asthma.

II. High Affinity IL-5 Monoclonal Antibodies

For use in constructing the antibodies, altered antibodies and fragments of this invention, a non-human species (for example, bovine, ovine, monkey, chicken, rodent (e.g., murine and rat), etc.) may be employed to generate a desirable immunoglobulin upon presentment with native human IL-5 or a peptide epitope therefrom. Conventional hybridoma techniques are employed to provide a hybridoma cell line secreting a non-human mAb to IL-5. Such hybridomas are then screened for binding using IL-5 coated to 96-well plates, as described in the Examples section, or alternatively with biotinylated IL-5 bound to a streptavidin coated plate.

One exemplary, high affinity, neutralizing mAb of this instant invention is mAb 2B6, a murine antibody which can be used for the development of a chimeric or humanized antibody, described in more detail in Example 1 below. The 2B6 mAb is characterized by an antigen binding specificity for human IL-5, with a K_a of less than 3.5×10^{-11} M (about 2.2×10^{-11} M) for IL-5. The K_a for IL-5 of a Fab fragment from 2B6 (see, Example 3H) is estimated to be about 9×10^{-11} M as determined by optical biosensor. MAb 2B6 appears to block the binding interaction between human IL-5 and the α-chain of the human IL-5 receptor.

Another desirable donor antibody is the murine mAb, 2E3. This mAb is characterized by being isotype IgG_{2a}, and having a dissociation constant for hIL-5 of less than 3.5×10^{-11} M (about 2.0×10^{-11} M).

Yet, another desirable donor antibody is the rat mAb, 4A6. This mAb is characterized by having a dissociation constant for hIL-5 of less than 3.5×10^{-11} M (about 1.8×10^{-11} M). In addition, mAb 4A6 appears to block the binding interaction between human IL-5 and the β-chain of the IL-5 receptor. MAb 4A6 does not block binding of human IL-5 to the α-chain of the IL-5 receptor. Thus, mAb 4A6 binds human IL-5 and an IL-5/IL-5 receptor α-chain complex.

This invention is not limited to the use of the 2B6 mAb, the 2E3 mAb, or its hypervariable (i.e., CDR) sequences. Any other appropriate high affinity IL-5 antibodies characterized by a dissociation constant equal or less than 3.5×10^{-11} M for human IL-5 and corresponding anti-IL-5 CDRs may be substituted therefor. Wherever

in the following description the donor antibody is identified as 2B6 or 2E3, this designation is made for illustration and simplicity of description only.

III. Antibody Fragments

The present invention also includes the use of Fab fragments or $F(ab')_2$ fragments derived from mAbs directed against human IL-5. These fragments are useful as agents protective *in vivo* against IL-5 and eosinophil-mediated conditions or *in vitro* as part of an IL-5 diagnostic. A Fab fragment contains the entire light chain and amino terminal portion of the heavy chain; and an $F(ab')_2$ fragment is the fragment formed by two Fab fragments bound by disulfide bonds. MAbs 2B6, 2E3, and other similar high affinity, IL-5 binding antibodies, provide sources of Fab fragments and $F(ab')_2$ fragments which can be obtained by conventional means, e.g., cleavage of the mAb with the appropriate proteolytic enzymes, papain and/or pepsin, or by recombinant methods. These Fab and $F(ab')_2$ fragments are useful themselves as therapeutic, prophylactic or diagnostic agents, and as donors of sequences including the variable regions and CDR sequences useful in the formation of recombinant or humanized antibodies as described herein.

The Fab and $F(ab')_2$ fragments can be constructed via a combinatorial phage library (see, e.g., Winter et al., Ann. Rev. Immunol., 12:433-455 (1994)) or via immunoglobulin chain shuffling (see, e.g., Marks et al., Bio/Technology, 10:779-783 (1992), which are both hereby incorporated by reference in their entirety) wherein the Fd or V_H immunoglobulin from a selected antibody (e.g., 2B6) is allowed to associate with a repertoire of light chain immunoglobulins, V_L (or V_K), to form novel Fabs. Conversely, the light chain immunoglobulin from a selected antibody may be allowed to associate with a repertoire of heavy chain immunoglobulins, V_H (or Fd), to form novel Fabs. Neutralizing IL-5 Fabs were obtained when the Fd of mAb 2B6 was allowed to associate with a repertoire of light chain immunoglobulins, as described in more detail in the Examples section. Hence, one is able to recover neutralizing Fabs with unique sequences (nucleotide and amino acid) from the chain shuffling technique.

IV. Anti-IL-5 Amino Acid and Nucleotide Sequences of Interest

The mAb 2B6 or other antibodies described above may contribute sequences, such as variable heavy and/or light chain peptide sequences, framework sequences, CDR sequences, functional fragments, and analogs thereof, and the nucleic acid sequences encoding them, useful in designing and obtaining various altered antibodies which are characterized by the antigen binding specificity of the donor antibody.

As one example, the present invention thus provides variable light chain and variable heavy chain sequences from the IL-5 murine antibody 2B6 and sequences derived therefrom. The heavy chain variable region of 2B6 is illustrated by FIG. 1.

The CDR-encoding regions are indicated by the boxed areas and are provided in SEQ ID NO: 7; SEQ ID NO: 8; and SEQ ID NO: 9. The light chain clone variable region of 2B6 is illustrated by FIG. 2. The CDR-encoding regions are provided in SEQ ID NO: 10; SEQ ID NO: 11; and SEQ ID NO: 12.

A humanized heavy chain variable region is illustrated in Fig. 8 [SEQ ID NOs: 18 and 19]. The signal sequence is also provided in SEQ ID NO: 17. Other suitable signal sequences, known to those of skill in the art, may be substituted for the signal sequences exemplified herein. The CDR amino acid sequences of this construct are identical to the native murine and chimeric heavy chain CDRs and are provided by SEQ ID NO: 7, SEQ ID NO: 8, and SEQ ID NO: 9. An exemplary (synthetic) humanized light chain variable sequence is illustrated in Fig. 9 [SEQ ID NOs: 20 and 21].

The nucleic acid sequences of this invention, or fragments thereof, encoding the variable light chain and heavy chain peptide sequences are also useful for mutagenic introduction of specific changes within the nucleic acid sequences encoding the CDRs or framework regions, and for incorporation of the resulting modified or fusion nucleic acid sequence into a plasmid for expression. For example, silent substitutions in the nucleotide sequence of the framework and CDR-encoding regions were used to create restriction enzyme sites which facilitated insertion of mutagenized CDR (and/or framework) regions. These CDR-encoding regions were used in the construction of a humanized antibody of this invention.

Taking into account the degeneracy of the genetic code, various coding sequences may be constructed which encode the variable heavy and light chain amino acid sequences, and CDR sequences of the invention as well as functional fragments and analogs thereof which share the antigen specificity of the donor antibody. The isolated nucleic acid sequences of this invention, or fragments thereof, encoding the variable chain peptide sequences or CDRs can be used to produce altered antibodies, e.g., chimeric or humanized antibodies, or other engineered antibodies of this invention when operatively combined with a second immunoglobulin partner.

It should be noted that in addition to isolated nucleic acid sequences encoding portions of the altered antibody and antibodies described herein, other such nucleic acid sequences are encompassed by the present invention, such as those complementary to the native CDR-encoding sequences or complementary to the modified human framework regions surrounding the CDR-encoding regions. Useful DNA sequences include those sequences which hybridize under stringent hybridization conditions [see, T. Maniatis et al, Molecular Cloning (A Laboratory Manual), Cold Spring Harbor Laboratory (1982), pages 387 to 389] to the DNA sequences. An

example of one such stringent hybridization condition is hybridization at 4XSSC at 65° C, followed by a washing in 0.1XSSC at 65°C for an hour. Alternatively an exemplary stringent hybridization condition is in 50% formamide, 4XSSC at 42°C. Preferably, these hybridizing DNA sequences are at least about 18 nucleotides in length, i.e., about the size of a CDR.

V. Altered immunoglobulin molecules and Altered antibodies

Altered immunoglobulin molecules can encode altered antibodies which include engineered antibodies such as chimeric antibodies and humanized antibodies. A desired altered immunoglobulin coding region contains CDR-encoding regions that encode peptides having the antigen specificity of an IL-5 antibody, preferably a high affinity antibody such as provided by the present invention, inserted into a first immunoglobulin partner (a human framework or human immunoglobulin variable region).

Preferably, the first immunoglobulin partner is operatively linked to a second immunoglobulin partner. The second immunoglobulin partner is defined above, and may include a sequence encoding a second antibody region of interest, for example an Fc region. Second immunoglobulin partners may also include sequences encoding another immunoglobulin to which the light or heavy chain constant region is fused in frame or by means of a linker sequence. Engineered antibodies directed against functional fragments or analogs of IL-5 may be designed to elicit enhanced binding with the same antibody.

The second immunoglobulin partner may also be associated with effector agents as defined above, including non-protein carrier molecules, to which the second immunoglobulin partner may be operatively linked by conventional means.

Fusion or linkage between the second immunoglobulin partners, e.g., antibody sequences, and the effector agent may be by any suitable means, e.g., by conventional covalent or ionic bonds, protein fusions, or hetero-bifunctional cross-linkers, e.g., carbodiimide, glutaraldehyde, and the like. Such techniques are known in the art and readily described in conventional chemistry and biochemistry texts.

Additionally, conventional linker sequences which simply provide for a desired amount of space between the second immunoglobulin partner and the effector agent may also be constructed into the altered immunoglobulin coding region. The design of such linkers is well known to those of skill in the art.

In addition, signal sequences for the molecules of the invention may be modified to enhance expression. As one example the 2B6 humanized antibody having the signal sequence and CDRs derived from the murine heavy chain sequence, had the original signal peptide replaced with another signal sequence [SEQ ID NO: 17].

An exemplary altered antibody contains a variable heavy and/or light chain peptide or protein sequence having the antigen specificity of mAb 2B6, e.g., the V_H and V_L chains. Still another desirable altered antibody of this invention is characterized by the amino acid sequence containing at least one, and preferably all of the CDRs of the variable region of the heavy and/or light chains of the murine antibody molecule 2B6 with the remaining sequences being derived from a human source, or a functional fragment or analog thereof. See, e.g., the humanized V_H and V_L regions (Figs. 8 and 9).

In still a further embodiment, the engineered antibody of the invention may have attached to it an additional agent. For example, the procedure of recombinant DNA technology may be used to produce an engineered antibody of the invention in which the Fc fragment or CH2 CH3 domain of a complete antibody molecule has been replaced by an enzyme or other detectable molecule (i.e., a polypeptide effector or reporter molecule).

The second immunoglobulin partner may also be operatively linked to a non-immunoglobulin peptide, protein or fragment thereof heterologous to the CDR-containing sequence having the antigen specificity of murine 2B6. The resulting protein may exhibit both anti-IL-5 antigen specificity and characteristics of the non-immunoglobulin upon expression. That fusion partner characteristic may be, e.g., a functional characteristic such as another binding or receptor domain, or a therapeutic characteristic if the fusion partner is itself a therapeutic protein, or additional antigenic characteristics.

Another desirable protein of this invention may comprise a complete antibody molecule, having full length heavy and light chains, or any discrete fragment thereof, such as the Fab or $F(ab')_2$ fragments, a heavy chain dimer, or any minimal recombinant fragments thereof such as an F_v or a single-chain antibody (SCA) or any other molecule with the same specificity as the selected donor mAb, e.g., mAb 2B6 or 2E3. Such protein may be used in the form of an altered antibody, or may be used in its unfused form.

Whenever the second immunoglobulin partner is derived from an antibody different from the donor antibody, e.g., any isotype or class of immunoglobulin framework or constant regions, an engineered antibody results. Engineered antibodies can comprise immunoglobulin (Ig) constant regions and variable framework regions from one source, e.g., the acceptor antibody, and one or more (preferably all) CDRs from the donor antibody, e.g., the anti-IL-5 antibody described herein. In addition, alterations, e.g., deletions, substitutions, or additions, of the acceptor mAb light and/or heavy variable domain framework region at the nucleic acid or amino acid levels, or

the donor CDR regions may be made in order to retain donor antibody antigen binding specificity.

Such engineered antibodies are designed to employ one (or both) of the variable heavy and/or light chains of the IL-5 mAb (optionally modified as described) or one or more of the below-identified heavy or light chain CDRs (see also FIG. 7). The engineered antibodies of the invention are neutralizing, i.e., they desirably block binding to the receptor of the IL-5 protein and they also block or prevent proliferation of IL-5 dependent cells.

Such engineered antibodies may include a humanized antibody containing the framework regions of a selected human immunoglobulin or subtype, or a chimeric antibody containing the human heavy and light chain constant regions fused to the IL-5 antibody functional fragments. A suitable human (or other animal) acceptor antibody may be one selected from a conventional database, e.g., the KABAT® database, Los Alamos database, and Swiss Protein database, by homology to the nucleotide and amino acid sequences of the donor antibody. A human antibody characterized by a homology to the framework regions of the donor antibody (on an amino acid basis) may be suitable to provide a heavy chain constant region and/or a heavy chain variable framework region for insertion of the donor CDRs. A suitable acceptor antibody capable of donating light chain constant or variable framework regions may be selected in a similar manner. It should be noted that the acceptor antibody heavy and light chains are not required to originate from the same acceptor antibody.

Desirably the heterologous framework and constant regions are selected from human immunoglobulin classes and isotypes, such as IgG (subtypes 1 through 4), IgM, IgA, and IgE. However, the acceptor antibody need not comprise only human immunoglobulin protein sequences. For instance a gene may be constructed in which a DNA sequence encoding part of a human immunoglobulin chain is fused to a DNA sequence encoding a non-immunoglobulin amino acid sequence such as a polypeptide effector or reporter molecule.

One example of a particularly desirable humanized antibody contains CDRs of 2B6 inserted onto the framework regions of a selected human antibody sequence. For neutralizing humanized antibodies, one, two or preferably three CDRs from the IL-5 antibody heavy chain and/or light chain variable regions are inserted into the framework regions of the selected human antibody sequence, replacing the native CDRs of the latter antibody.

Preferably, in a humanized antibody, the variable domains in both human heavy and light chains have been engineered by one or more CDR replacements. It is possible to use all six CDRs, or various combinations of less than the six CDRs.

Preferably all six CDRs are replaced. It is possible to replace the CDRs only in the human heavy chain, using as light chain the unmodified light chain from the human acceptor antibody. Still alternatively, a compatible light chain may be selected from another human antibody by recourse to the conventional antibody databases. The remainder of the engineered antibody may be derived from any suitable acceptor human immunoglobulin.

The engineered humanized antibody thus preferably has the structure of a natural human antibody or a fragment thereof, and possesses the combination of properties required for effective therapeutic use, e.g., treatment of IL-5 mediated inflammatory diseases in man, or for diagnostic uses.

As another example, an engineered antibody may contain three CDRs of the variable light chain region of 2E3 [SEQ ID NO: 10, 11 and 13] and three CDRs of the variable heavy chain region of 2B6 [SEQ ID NO: 7, 8 and 9]. The resulting humanized antibody should be characterized by the same antigen binding specificity and high affinity of mAb 2B6.

It will be understood by those skilled in the art that an engineered antibody may be further modified by changes in variable domain amino acids without necessarily affecting the specificity and high affinity of the donor antibody (i.e., an analog). It is anticipated that heavy and light chain amino acids may be substituted by other amino acids either in the variable domain frameworks or CDRs or both.

In addition, the constant region may be altered to enhance or decrease selective properties of the molecules of the instant invention. For example, dimerization, binding to Fc receptors, or the ability to bind and activate complement (see, e.g., Angal et al., Mol. Immunol, 30:105-108 (1993), Xu et al., J. Biol. Chem, 269:3469-3474 (1994), Winter et al., EP 307,434-B).

An altered antibody which is a chimeric antibody differs from the humanized antibodies described above by providing the entire non-human donor antibody heavy chain and light chain variable regions, including framework regions, in association with human immunoglobulin constant regions for both chains. It is anticipated that chimeric antibodies which retain additional non-human sequence relative to humanized antibodies of this invention may elicit a significant immune response in humans.

Such antibodies are useful in the prevention and treatment of IL-5 mediated disorders, as discussed below.

VI. Production of Altered antibodies and Engineered Antibodies

Preferably, the variable light and/or heavy chain sequences and the CDRs of mAb 2B6 or other suitable donor mAbs (e.g., 2E3, 2F2, 4A6, etc.), and their encoding nucleic acid sequences, are utilized in the construction of altered antibodies, preferably

humanized antibodies, of this invention, by the following process. The same or similar techniques may also be employed to generate other embodiments of this invention.

A hybridoma producing a selected donor mAb, e.g., the murine antibody 2B6, is conventionally cloned, and the DNA of its heavy and light chain variable regions obtained by techniques known to one of skill in the art, e.g., the techniques described in Sambrook *et al.*, (Molecular Cloning (A Laboratory Manual), 2nd edition, Cold Spring Harbor Laboratory (1989)). The variable heavy and light regions of 2B6 containing at least the CDR-encoding regions and those portions of the acceptor mAb light and/or heavy variable domain framework regions required in order to retain donor mAb binding specificity, as well as the remaining immunoglobulin-derived parts of the antibody chain derived from a human immunoglobulin are obtained using polynucleotide primers and reverse transcriptase. The CDR-encoding regions are identified using a known database and by comparison to other antibodies.

A mouse/human chimeric antibody may then be prepared and assayed for binding ability. Such a chimeric antibody contains the entire non-human donor antibody V_H and V_L regions, in association with human Ig constant regions for both chains.

Homologous framework regions of a heavy chain variable region from a human antibody were identified using computerized databases, e.g., KABAT®, and a human antibody having homology to 2B6 was selected as the acceptor antibody. The sequences of synthetic heavy chain variable regions containing the 2B6 CDR-encoding regions within the human antibody frameworks were designed with optional nucleotide replacements in the framework regions to incorporate restriction sites. This designed sequence was then synthesized using long synthetic oligomers. Alternatively, the designed sequence can be synthesized by overlapping oligonucleotides, amplified by polymerase chain reaction (PCR), and corrected for errors.

A suitable light chain variable framework region was designed in a similar manner.

A humanized antibody may be derived from the chimeric antibody, or preferably, made synthetically by inserting the donor mAb CDR-encoding regions from the heavy and light chains appropriately within the selected heavy and light chain framework. Alternatively, a humanized antibody of the invention made be prepared using standard mutagenesis techniques. Thus, the resulting humanized antibody contains human framework regions and donor mAb CDR-encoding regions. There may be subsequent manipulation of framework residues. The resulting humanized antibody can be expressed in recombinant host cells, e.g., COS, CHO or myeloma

cells. Other humanized antibodies may be prepared using this technique on other suitable IL-5-specific, neutralizing, high affinity, non-human antibodies.

A conventional expression vector or recombinant plasmid is produced by placing these coding sequences for the altered antibody in operative association with conventional regulatory control sequences capable of controlling the replication and expression in, and/or secretion from, a host cell. Regulatory sequences include promoter sequences, e.g., CMV promoter, and signal sequences, which can be derived from other known antibodies. Similarly, a second expression vector can be produced having a DNA sequence which encodes a complementary antibody light or heavy chain. Preferably this second expression vector is identical to the first except insofar as the coding sequences and selectable markers are concerned, so to ensure as far as possible that each polypeptide chain is functionally expressed. Alternatively, the heavy and light chain coding sequences for the altered antibody may reside on a single vector.

A selected host cell is co-transfected by conventional techniques with both the first and second vectors (or simply transfected by a single vector) to create the transfected host cell of the invention comprising both the recombinant or synthetic light and heavy chains. The transfected cell is then cultured by conventional techniques to produce the engineered antibody of the invention. The humanized antibody which includes the association of both the recombinant heavy chain and/or light chain is screened from culture by appropriate assay, such as ELISA or RIA. Similar conventional techniques may be employed to construct other altered antibodies and molecules of this invention.

Suitable vectors for the cloning and subcloning steps employed in the methods and construction of the compositions of this invention may be selected by one of skill in the art. For example, the conventional pUC series of cloning vectors, may be used. One vector used is pUC19, which is commercially available from supply houses, such as Amersham (Buckinghamshire, United Kingdom) or Pharmacia (Uppsala, Sweden). Additionally, any vector which is capable of replicating readily, has an abundance of cloning sites and selectable genes (e.g., antibiotic resistance), and is easily manipulated may be used for cloning. Thus, the selection of the cloning vector is not a limiting factor in this invention.

Similarly, the vectors employed for expression of the engineered antibodies according to this invention may be selected by one of skill in the art from any conventional vector. The vectors also contain selected regulatory sequences (such as CMV promoters) which direct the replication and expression of heterologous DNA sequences in selected host cells. These vectors contain the above described DNA

sequences which code for the engineered antibody or altered immunoglobulin coding region. In addition, the vectors may incorporate the selected immunoglobulin sequences modified by the insertion of desirable restriction sites for ready manipulation.

The expression vectors may also be characterized by genes suitable for amplifying expression of the heterologous DNA sequences, e.g., the mammalian dihydrofolate reductase gene (DHFR). Other preferable vector sequences include a poly A signal sequence, such as from bovine growth hormone (BGH) and the betaglobin promoter sequence (betaglopro). The expression vectors useful herein may be synthesized by techniques well known to those skilled in this art.

The components of such vectors, e.g. replicons, selection genes, enhancers, promoters, signal sequences and the like, may be obtained from commercial or natural sources or synthesized by known procedures for use in directing the expression and/or secretion of the product of the recombinant DNA in a selected host. Other appropriate expression vectors of which numerous types are known in the art for mammalian, bacterial, insect, yeast, and fungal expression may also be selected for this purpose.

The present invention also encompasses a cell line transfected with a recombinant plasmid containing the coding sequences of the engineered antibodies or altered immunoglobulin molecules thereof. Host cells useful for the cloning and other manipulations of these cloning vectors are also conventional. However, most desirably, cells from various strains of *E. coli* are used for replication of the cloning vectors and other steps in the construction of altered antibodies of this invention.

Suitable host cells or cell lines for the expression of the engineered antibody or altered antibody of the invention are preferably mammalian cells such as CHO, COS, a fibroblast cell (e.g., 3T3), and myeloid cells, and more preferably a CHO or a myeloid cell. Human cells may be used, thus enabling the molecule to be modified with human glycosylation patterns. Alternatively, other eukaryotic cell lines may be employed. The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. See, e.g., Sambrook *et al.*, cited above.

Bacterial cells may prove useful as host cells suitable for the expression of the recombinant Fabs of the present invention (see, e.g., Plückthun, A., Immunol. Rev., 130:151-188 (1992)). However, due to the tendency of proteins expressed in bacterial cells to be in an unfolded or improperly folded form or in a non-glycosylated form, any recombinant Fab produced in a bacterial cell would have to be screened for retention of antigen binding ability. If the molecule expressed by the bacterial cell was produced in a properly folded form, that bacterial cell would be a desirable host. For

example, various strains of *E. coli* used for expression are well-known as host cells in the field of biotechnology. Various strains of *B. subtilis*, *Streptomyces*, other bacilli and the like may also be employed in this method.

Where desired, strains of yeast cells known to those skilled in the art are also available as host cells, as well as insect cells, e.g. *Drosophila* and *Lepidoptera* and viral expression systems. See, e.g. Miller *et al.*, Genetic Engineering, 8:277-298, Plenum Press (1986) and references cited therein.

The general methods by which the vectors of the invention may be constructed, the transfection methods required to produce the host cells of the invention, and culture methods necessary to produce the altered antibody of the invention from such host cell are all conventional techniques. Likewise, once produced, the altered antibodies of the invention may be purified from the cell culture contents according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like. Such techniques are within the skill of the art and do not limit this invention.

Yet another method of expression of the humanized antibodies may utilize expression in a transgenic animal, such as described in U. S. Patent No. 4,873,316. This relates to an expression system using the animal's casein promoter which when transgenically incorporated into a mammal permits the female to produce the desired recombinant protein in its milk.

Once expressed by the desired method, the engineered antibody is then examined for *in vitro* activity by use of an appropriate assay. Presently conventional ELISA assay formats are employed to assess qualitative and quantitative binding of the engineered antibody to IL-5. Additionally, other *in vitro* assays may also be used to verify neutralizing efficacy prior to subsequent human clinical studies performed to evaluate the persistence of the engineered antibody in the body despite the usual clearance mechanisms.

Following the procedures described for humanized antibodies prepared from 2B6, one of skill in the art may also construct humanized antibodies from other donor IL-5 antibodies, variable region sequences and CDR peptides described herein. Engineered antibodies can be produced with variable region frameworks potentially recognized as "self" by recipients of the engineered antibody. Minor modifications to the variable region frameworks can be implemented to effect large increases in antigen binding without appreciable increased immunogenicity for the recipient. Such engineered antibodies may effectively treat a human for IL-5 mediated conditions. Such antibodies may also be useful in the diagnosis of such conditions.

VII. Therapeutic/Prophylactic/Diagnostic Uses

This invention also relates to a method of treating humans experiencing eosinophilia-related symptoms, i.e., conditions associated with excess eosinophil production, such as asthma, which comprises administering an effective dose of antibodies including one or more of the engineered antibodies or altered antibodies described herein, or fragments thereof.

The therapeutic response induced by the use of the molecules of this invention is produced by binding to human IL-5 and thus subsequently blocking eosinophil stimulation. Preferably, the molecules of this invention are non-competitive with the IL-5 receptor alpha-chain for binding human IL-5. That is, the preferred molecules of this invention do not block the binding of human IL-5 to the α-chain of the human IL-5 receptor. Thus, the molecules of the present invention, when in preparations and formulations appropriate for therapeutic use, are highly desirable for those persons experiencing an allergic and/or atopic response, or a response associated with eosinophilia, such as but not limited to, allergic rhinitis, asthma, chronic eosinophilic pneumonia, allergic bronchopulmonary aspergillosis, coeliac disease, eosinophilic gastroenteritis, Churg-Strauss syndrome (periarteritis nodosa plus atopy), eosinophilic myalgia syndrome, hypereosinophilic syndrome, oedematous reactions including episodic angiodema, helminth infections, where eosinophils may have a protective role, onchocercal dermatitis and atopic dermatitis.

The altered antibodies, antibodies and fragments thereof of this invention may also be used in conjunction with other antibodies, particularly human mAbs reactive with other markers (epitopes) responsible for the condition against which the engineered antibody of the invention is directed.

The therapeutic agents of this invention are believed to be desirable for treatment of allergic conditions from about 2 days to about 3 weeks, or as needed. For example, longer treatments may be desirable when treating seasonal rhinitis or the like. This represents a considerable advance over the currently used infusion protocol with prior art treatments of IL-5 mediated disorders. The dose and duration of treatment relates to the relative duration of the molecules of the present invention in the human circulation, and can be adjusted by one of skill in the art depending upon the condition being treated and the general health of the patient.

The mode of administration of the therapeutic agent of the invention may be any suitable route which delivers the agent to the host. The altered antibodies, antibodies, engineered antibodies, and fragments thereof, and pharmaceutical compositions of the invention are particularly useful for parenteral administration, i.e., subcutaneously, intramuscularly, intravenously, or intranasally.

Therapeutic agents of the invention may be prepared as pharmaceutical compositions containing an effective amount of the engineered (e.g., humanized) antibody of the invention as an active ingredient in a pharmaceutically acceptable carrier. In the prophylactic agent of the invention, an aqueous suspension or solution containing the engineered antibody, preferably buffered at physiological pH, in a form ready for injection is preferred. The compositions for parenteral administration will commonly comprise a solution of the engineered antibody of the invention or a cocktail thereof dissolved in an pharmaceutically acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be employed, e.g., 0.4% saline, 0.3% glycine, and the like. These solutions are sterile and generally free of particulate matter. These solutions may be sterilized by conventional, well known sterilization techniques (e.g., filtration). The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, etc. The concentration of the antibody of the invention in such pharmaceutical formulation can vary widely, i.e., from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., according to the particular mode of administration selected.

Thus, a pharmaceutical composition of the invention for intramuscular injection could be prepared to contain 1 mL sterile buffered water, and between about 1 ng to about 100 mg, e.g. about 50 ng to about 30 mg or more preferably, about 5 mg to about 25 mg, of an engineered antibody of the invention. Similarly, a pharmaceutical composition of the invention for intravenous infusion could be made up to contain about 250 ml of sterile Ringer's solution, and about 1 to about 30 and preferably 5 mg to about 25 mg of an engineered antibody of the invention. Actual methods for preparing parenterally administrable compositions are well known or will be apparent to those skilled in the art and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pennsylvania.

It is preferred that the therapeutic agent of the invention, when in a pharmaceutical preparation, be present in unit dose forms. The appropriate therapeutically effective dose can be determined readily by those of skill in the art. To effectively treat an inflammatory disorder in a human or other animal, one dose of approximately 0.1 mg to approximately 20 mg per 70 kg body weight of a protein or an antibody of this invention should be administered parenterally, preferably *i.v.* or i.m. (intramuscularly). Such dose may, if necessary, be repeated at appropriate time intervals selected as appropriate by a physician during the inflammatory response.

The antibodies, altered antibodies and engineered antibodies of this invention may also be used in diagnostic regimens, such as for the determination of IL-5 mediated disorders or tracking progress of treatment of such disorders. As diagnostic reagents, these antibodies may be conventionally labeled for use in ELISA's and other conventional assay formats for the measurement of IL-5, and/or IL-5/IL-5 receptor α-chain complex levels in serum, plasma or other appropriate tissue, or the release by human cells in culture. The nature of the assay in which the altered antibodies are used are conventional and do not limit this disclosure.

Thus, one embodiment of the present invention relates to a method for aiding the diagnosis of allergies and other conditions associated with excess eosinophil production in a patient which comprises the steps of determining the amount of human IL-5 and/or IL-5/IL-5 receptor α-chain complex in sample (plasma or tissue) obtained from said patient and comparing said determined amount to the mean amount of human IL-5 in the normal population, whereby the presence of a significantly elevated amount of IL-5 and/or IL-5/IL-5 receptor α-chain complex in the patient's sample is an indication of allergies and other conditions associated with excess eosinophil production.

In the compound screening embodiment of this invention, the human IL-5 receptor β-chain is isolated in a membrane fraction, or in cell bound form, and is contacted with a plurality of candidate molecules from which candidates are selected which bind to and interact with the receptor. The candidate compounds can be subjected to a competition screening assays, in which a known ligand, i.e, human IL-5 or IL-5/IL-5 receptor α-chain complex, preferably labeled with an analytically detectable reagent, most preferably radioactivity, is introduced with the drug to be tested and the compound's capacity to inhibit or enhance the binding of the labeled ligand is measured. Alternatively, the binding or interaction can be measured directly by using radioactively labeled candidate compounds of interest or by the second messenger effect resulting from the interaction or binding of the candidate compounds. Compounds are screened for their increased affinity and selectivity to the receptor interest. Molecules that bind gratuitously, i.e., without inducing effects on the human IL-5 receptor β-chain, are most likely to be good antagonists. Potential antagonists include small organic molecules, peptides, polypeptides and antibodies specific for the IL-5 receptor β-chain and thereby inhibit or extinguish its activity.

The antibodies, altered antibodies or fragments thereof described herein can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional immunoglobulins and art-known lyophilization and reconstitution techniques can be employed.

The following examples illustrate various aspects of this invention including the construction of exemplary engineered antibodies and expression thereof in suitable vectors and host cells, and are not to be construed as limiting the scope of this invention. All amino acids are identified by conventional three letter or single letter codes. All necessary restriction enzymes, plasmids, and other reagents and materials were obtained from commercial sources unless otherwise indicated. All general cloning ligation and other recombinant DNA methodology were as performed in T. Maniatis *et al.*, cited above, or the second edition thereof (1989), eds. Sambrook *et al.*, by the same publisher ("Sambrook *et al.*").

Example 1 - Production of MAbs to hIL-5

Human IL-5 was expressed in *Drosophila* Schneider 2 (S_2) cells and purified to homogeneity. Murine IL-5 was expressed in Baculovirus using *Spodoptera frugiperda* 21 (Sf21) cells and purified to homogeneity. Monoclonal antibody TRFK-5 (a neutralizing rat anti-mouse IL-5 antibody) was obtained from Genzyme Corp. (Cambridge, MA).

A. *Immunization Procedure*:

Recombinant human IL-5 (IL-5) was used as the immunogen for a panel of seven CAF1 female mice (Charles River, Wilmington, MA). The animals received three subcutaneous injections of IL-5 in phosphate buffered saline (PBS) emulsified with a one to one ratio of TiterMAX™ (CytoRx Corp., Norcross, GA) over a period of four months. The priming antigen dose was 50 µg (micrograms) and boosts were 25 and 10 µg (micrograms). After the boosts, serum samples were collected and assayed both for binding to IL-5 and for neutralization activity via the receptor binding inhibition assay and B13 proliferation assay (or IL-5 neutralization assay (Example 2C)). All of the mice produced serum samples that bound to IL-5. Animals selected as spleen donors were boosted intravenously with 10 µg (micrograms) of recombinant human IL-5 three days prior to euthanasia.

B. *Hybridoma Development*:

The fusion procedure, first reported by Kohler et al., (Nature, 256:495 (1975)), was used with modifications to perform the technique using a cell monolayer (Kennet et al., Eds., "Hybridomas: A new dimension in biological analysis", pp. 368-377, Plenum Press, New York). Spleen cells from two donor mice were pooled and fusions performed using a ratio of 50 million spleen cells to ten million SP2/0/Ag14 myeloma cells. Supernatants from fusion-positive wells were assayed for binding to IL-5 by ELISA. Wells containing cells producing antibody to IL-5 were expanded and supernatants screened in an IL-5 receptor binding inhibition assay, and a B13 (neutralization) proliferation assay (described below).

Sixteen hybridomas were isolated which secreted mAbs reactive with IL-5. The hybridoma supernatants were mixed with iodinated IL-5, added to a membrane extract prepared from *Drosophila* cells expressing the α-chain of the IL-5 receptor (IL-5R), and assayed for inhibition of receptor binding. Eleven of the hybridoma supernatants inhibited by greater than 60% the binding of iodinated IL-5 to the IL-5 receptor α-chain. Three of the mAbs, 2B6, 2E3 and 2F2, also inhibited by greater than 70% the proliferation of murine B13 cells in response to human but not murine IL-5. Five of the hybridomas, four of which blocked binding and/or proliferation (1C6, 2B6, 2E3 and 2F2) and 1 of which was non-neutralizing (24G9), were repeatedly subcloned

in soft agar to generate stable clonal cell lines. Supernatants from the cloned lines were screened for cross-reactivity by ELISA and did not bind to human IL-1α, IL-1β, IL-4, IL-8, M-CSF or TGFα. The mAbs were purified and binding affinities were estimated from optical biosensor (BIAcore) analysis to range from 10 to 100 pM. Supernatants from the lines were isotyped using murine isotyping reagents (PharMingen, San Diego, CA). A summary of the affinities and IC_{50} for neutralizing activities of the mAbs is presented in Table I (Example 2).

By similar methods, rat hybridomas were derived from immunized rats, using a comparable immunization protocol and rat myelomas for the fusion as described for the mouse. Two rat hybridomas, 4A6 and 5D3, were identified that produced mAbs which bound to IL-5. Like mAbs 2B6, 2E3 and 2F2, mAbs 4A6 and 5D3 were found to be neutralizing in the B13 assay described below.

C. *Hybridoma Deposit:*

The hybridoma cell line SK119-2B6.206.75(1) producing monoclonal antibody 2B6 was deposited with the American Type Culture Collection (ATCC), Rockville, MD, USA, under accession number HB 11783, and has been accepted as a patent deposit, in accordance with the Budapest Treaty of 1977 governing the deposit of microorganisms for the purposes of patent procedure.

The hybridoma cell line SK119-2E3.39.40.2 producing monoclonal antibody 2E3 was deposited with the American Type Culture Collection (ATCC), Rockville, MD, USA, under accession number HB 11782, and has been accepted as a patent deposit, in accordance with the Budapest Treaty of 1977 governing the deposit of microorganisms for the purposes of patent procedure.

The hybridoma cell line SK119-2F2.37.80.12 producing monoclonal antibody 2F2 was deposited with the American Type Culture Collection (ATCC), Rockville, MD, USA, under accession number HB 11781, and has been accepted as a patent deposit, in accordance with the Budapest Treaty of 1977 governing the deposit of microorganisms for the purposes of patent procedure.

The hybridoma cell line SK119-24G9.8.20.5 producing monoclonal antibody 24G9 was deposited with the American Type Culture Collection (ATCC), Rockville, MD, USA, under accession number HB 11780, and has been accepted as a patent deposit, in accordance with the Budapest Treaty of 1977 governing the deposit of microorganisms for the purposes of patent procedure.

The hybridoma cell line 4A6(1)G1F7 producing monoclonal antibody 4A6 was deposited with the American Type Culture Collection (ATCC), Rockville, MD, USA, under accession number HB 11943, and has been accepted as a patent deposit, in

accordance with the Budapest Treaty of 1977 governing the deposit of microorganisms for the purposes of patent procedure.

The hybridoma cell line 5D3(1)F5D6 producing monoclonal antibody 5D3 was deposited with the American Type Culture Collection (ATCC), Rockville, MD, USA, under accession number HB 11942, and has been accepted as a patent deposit, in accordance with the Budapest Treaty of 1977 governing the deposit of microorganisms for the purposes of patent procedure.

Example 2 - Assays

A. *ELISA*:

Individual wells of MaxiSorb™ immuno plates (Nunc, Naperville, IL) were coated with 0.2 ug IL-5 in 0.05M carbonate buffer pH 9.6. After incubating overnight at 4°C, the plates were rinsed with PBS containing 0.025% Tween® 20, and blocked with 1% BSA in PBS with 0.025% Tween® 20 for two hours at room temperature. Undiluted hybrid supernatants were added to the IL-5 coated wells and incubated at room temperature for two hours. After the plates were rinsed, peroxidase labeled goat anti-mouse IgG & IgM (Boehringer Mannheim, Indianapolis, IN) was added at 1/7500 dilution in PBS containing 1% BSA and 0.025% Tween® 20. Two hours later the plates were washed and 0.2 ml of 0.1M citrate buffer pH 4.75 containing 0.1% urea peroxide and 1mg/ml orthophenylenediamine was added. After 15 min the plates were read at 450nm on a VMax™ Microplate Reader (Molecular Devices, Menlo Park, CA).

B. *Receptor Binding Inhibition Assay*:

Membrane extracts of *Drosophila* S2 cells expressing the α-chain of the human IL-5 Receptor (IL-5R) were used to measure the effect of antibody on IL-5 binding to receptor. To prepare the membranes, 10^9 cells were pelleted at 1000 x g at 4°C for 10 min. The cell pellet was frozen in a dry ice/ethanol bath for 15 min. The pellet was thawed, resuspended in 10 ml PBS at 4°C and pelleted at 1000 x g for 10 min. The cell pellet was washed 2X in PBS and resuspended in 13.5 ml Hypotonic buffer (10 mM Tris pH 7.5, 3 mM $MgCl_2$, 1 mM dithiothreitol, 1mM phenylmethylsulfonyl fluoride, 1 uM leupeptin, 1 uM pepstatin A) and incubated on ice for 5 min. The cell suspension was homogenized in a 15 ml Dounce homogenizer and brought to a final concentration of 0.25 M sucrose with a solution of 2.5 M sucrose. Cell debris was removed by a 15 min centrifugation at 1000 x g. Cell membranes were pelleted at 100,000 x g at 4°C for 90 min and resuspended in 50 ml of 10 mM Tris pH 7.5, 3 mM $MgCl_2$, 250 mM sucrose, and stored at -70°C.

Assays with *Drosophila* membranes containing receptor were performed in MultiscreenGV™ plates (Millipore Corp., Bedford, MA) using *Drosophila* tissue

culture medium M3 (Lindquist et al., Drosophila Inf. Serv., 58: 163 (1982)) containing 25 mM HEPES buffer pH 7.2 and 0.1% BSA (Binding Buffer). Wells were pre-blocked with 0.1 ml binding buffer. 50 ul of the test sample, in triplicate, was added to wells followed by 25 ul iodinated (^{125}I) IL-5. After 20 minutes incubation at room temperature, 25 ul of the membrane extract of *Drosophila* S2 cells expressing the α-chain of the human IL5R was added to the wells. After 1 hour further incubation the membranes were collected by vacuum filtration and washed 3X with binding buffer. Filters were dried and counted.

C. *IL-5 Neutralization Assay*:

The murine IL-5/IL-3 dependent cell line LyH7.B13 (B13) was obtained courtesy of R. Palacios, Basel Institute of Immunology, Switzerland. Cells were subcultured twice weekly in RPMI 1640 medium (GibcoBRL, Renfrewshire, UK), supplemented with L-Glutamine, non-essential amino acids, sodium pyruvate, penicillin-streptomycin (all GibcoBRL), plus 2-mercaptoethanol (5×10^{-5} M, Sigma), 10% fetal bovine serum (Globepharm, Surrey, UK) and 1-10 units murine IL-5. For assays, cells were cultured for 48 hours in triplicate (5000 cells/well) in 96-well round bottom plates in the presence of appropriately diluted test samples and pulsed with 0.5 uCi ^{3}H-thymidine (Amersham, Bucks, UK) for the final 4 hours. They were processed for scintillation counting in a 1205 Betaplate (LKB Wallac, Beds, UK).

D. *Optical Biosensor*:

Kinetic and equilibrium binding properties with immobilized hIL-5 and antibodies were measured using a BIAcore optical biosensor (Pharmacia Biosensor, Uppsala, Sweden). Kinetic data were evaluated using relationships described previously (Karlsson et al., J. Immunol. Meth., 145:229-240 (1991)) and which is incorporated by reference in its entirety.

Three of the neutralizing mAbs, namely 2B6, 2E3 and 2F2, had very similar potencies of inhibition of ^{125}I-IL-5 binding to membrane receptor and neutralization of B cell proliferation and also very similar affinities for IL-5 (see Table I). The nucleotide sequences of the V_H and V_L from these three mAbs, 2 IgG1 and 1 IgG2a, respectively, were determined. The sequences obtained were very similar, differing only at a few residues.

TABLE I

Affinity and neutralizing activity of mAbs reactive with human IL-5

mAb	Kd (pM)[a]	Neutralization		
		Binding IC_{50}(nM)[b]	Proliferation IC_{50}[c]	100%Inhibition[c]
2B6	22	1	70	200
2E3	20	1	90	600
2F2	13	1	150	340
1C6	86	43	12,200	ND
24G9	ND	>133	>100,000	ND
4A6	18	>88	28	100
5D3	ND	ND	100	10,000

[a] Determined by optical biosensor (BIAcore) analysis (25°C)
[b] Inhibition of ^{125}I-IL-5 binding to IL-5R(α chain) from *Drosophila* membranes
[c] Inhibition of proliferation (in pM) of B13 cells in response to 8 pM human IL-5
ND = No data

Example 3-Isolation and Characterization of IL-5 Fabs from Combinatorial Library

A. *PCR and Combinatorial Library Construction*:

RNA purified from the spleens of three mice was reverse transcribed with a cDNA kit (Boehringer Mannheim, Indianapolis, IN) using either the primer (dT)15 supplied with the kit or the 3' Fd (IgG1, IgG2a & IgG3) and kappa light chain primers as described by Huse et al. (Science, 246:1275 (1989)) and Kang, S.A. (Methods: Companion Methods Enzymol., 2:111 (1991)) which are hereby incorporated by reference in their entirety. Immunoglobulin cDNAs were amplified by PCR using the primers and the thermal cycling conditions described (Huse et al. *supra*). The *Hot Start* technique using AmpliWax™ PCR Gem 100 (Perkin Elmer Cetus, Norwalk, CT) beads and the manufacturer's protocol was used for all of the reactions. The PCR products were gel purified, digested, and ligated into the pMKFabGene3 vector (Ames et al., J. Immunol., 152:4572 (1994)). The library titer following ligation with the Fd cDNAs was 5.1 X 10^7 CFU and following ligation with the kappa cDNAs was 1.5 X 10^6 CFU. XL1-Blue cells (Stratagene, La Jolla, CA) transformed with the phagemid library were infected with helper phage VCSM13 (Stratagene) and phage were prepared as described by Barbas and Lerner (Methods: Companion Methods Enzymol., 2:119 (1991)).

B. *Biopanning*:

Four microtiter wells (Immulon II Removawell Strips, Dynatech Laboratories Inc., Chantilly, VA) were coated overnight at 4°C with IL-5 (1ug/well) in 0.1M bicarbonate, pH 8.6. The wells were washed with water and blocked with PBS containing 3% BSA at 37°C for 1 hour. The blocking solution was removed, and the library was added to microtiter wells (50 ul/well) and incubated at 37°C for 2 hours. Wells were washed 10 times with TBS/Tween® (50mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.5% Tween® 20) and once with H_2O prior to elution of the adherent phage with 0.1 M HCl, adjusted to pH 2.2 with glycine, containing 1 mg/ml BSA.

C. *Colony Lifts*:

Colony lifts from clones isolated from the third and fourth rounds of biopanning were processed as described (Barbas and Lerner, *supra*). Filters were incubated for 1 hour at room temperature with 0.5-1.0 uCi ^{125}I-IL-5, which had been iodinated using Bolton-Hunter reagent (NEN, Billerica, MA) following the manufacturers recommended procedure, in PBS containing 1% BSA, washed with PBS 0.25% Tween, and exposed to Kodak XAR film. Colonies expressing IL-5-reactive Fabs were detected by autoradiography.

D. *Preparation of Soluble FABs*:

Phagemid DNAs were digested with NheI and SpeI to remove gene III and self-ligated. XL1-Blue cells were transformed, and isolated clones were grown overnight at 37°C in 5.0 ml super broth (SB) medium (30 g tryptone, 20 g yeast extract, 10 g 3-[N-Morpholino]propanesulfonic acid, MOPS with pH adjusted to 7) containing 1% glucose and 50 ug/ml carbenicillin. Cells from 1 ml of this culture were pelleted at 3500 rpm for 10 min in Beckman GS-6R centrifuge and used to inoculate 5 ml SB containing 50 ug/ml carbenicillin. Cultures were shaken for 1 hour at 37°C, Isopopyl-b-D-thiogalactopyranoside (IPTG; 1 mM) was added and the cultures were transferred to 28°C overnight. Soluble Fab was prepared from periplasmic extracts by lysing the cell pellet for 20 min at 4°C in 20% sucrose suspended in 30 mM Tris pH 8.0, followed by centrifugation in a Microfuge for 10 min. Fab concentrations were estimated by western blot by comparison to samples containing known amounts of murine Fab. The different bacterial periplasmic extracts contained similar concentrations of Fab, ranging from 1 to 20 ug/ml, as estimated by western blot analysis.

E. *Purification of FABs*:

A chelating peptide was engineered onto the carboxy-terminal end of the heavy chain to aid in protein purification. Following removal of the M13 geneIII coding region, via digestion with NheI and SpeI, a pair of overlapping oligonucleotides: [SEQ ID NO: 43] 5'-CTAGCCACCACCACCACCACCACTAA-3';

[SEQ ID NO: 44] 3'-GGTGGTGGTGGTGGTGATTGATC-5'
encoding six histidine residues were subcloned into the Fab expression vector. Induction of Fab expression was performed as described above. Following overnight induction at 28°C periplasmic lysate of the cell pellet was prepared by 30 min incubation at 4°C in 20% sucrose, 30 mM TRIS pH 8.0. Urea and Brij-35 detergent were added to the clarified supernatant to final concentrations of 2M and 1% respectively. After stirring at room temperature for 1 hour, the treated and clarified supernatant was loaded at 0.5 ml/min directly onto a 5 ml Nickel-NTA metal chelating column (1.5 x 3 cm) equilibrated with buffer A (100 mM Na-Phosphate, 10 mM Tris, 0.3 M NaCl, 2 M urea, pH 8.0). After a 4 column volume (20 ml) wash bound materials were eluted with a 6 column volume (30 ml) reverse pH gradient from pH 8 to pH 4 in the same buffer as above. The purified Fabs eluted from the column in a sharp symmetrical peak at pH 5.5. They were >90% pure and free of DNA.

F. *FAB ELISA*:

Immulon II plates (Dynatech) were coated overnight at 4°C with protein suspended (1 mg/ml; 50 ml per well) in 0.1 M bicarbonate buffer, pH 8.6. Dilutions and washes were performed in PBS containing 0.05% Tween™ 20. Plates were washed and blocked for 1 hour with PBS containing 1% BSA at room temperature. Various dilutions of the bacterial supernatants containing soluble Fabs, or purified Fabs, were added to the plates. Following a one hour incubation plates were washed and biotinylated goat anti-mouse kappa (Southern Biotechnology Associates, Inc., Birmingham, AL) was added (1:2000 dilution; 50 ul/well) for 1 hour. The plates were washed and streptavidin labeled horseradish peroxidase was added (1:2000 dilution; 50 ul/well) for 1 hour. The plates were washed, ABTS peroxidase substrate was added (100 ul/well; Kirkegaard & Perry Laboratories, Gaithersburg, MD) and the optical density at 405 nm was read on a UVmax™ (Molecular Devices) microplate reader.

G. *Isolation and Characterization of Fabs from a Combinatorial Library*:

Phage bearing Fabs to IL-5 were selected from the library by multiple rounds of biopanning against microtiter wells coated with IL-5. After 4 rounds of selection IL-5 reactive Fabs were identified by a colony lift assay using ^{125}I-IL-5. Thirty four colonies from the third round and 4 colonies from the fourth round were identified which bound labeled IL-5. Binding to IL-5 was confirmed by direct binding ELISA using culture supernatants expressing the Fab-geneIII fusion protein. DNA was isolated from these colonies and, after removing the coding region of M13 gene III, soluble Fab expression was induced. Periplasmic fractions were prepared and assayed by ELISA for binding to IL-5. The Fabs bound specifically to IL-5 with no demonstrable binding to an another protein, rC5a.

The undiluted periplasmic extracts (containing 1 to 20 ug/ml Fab) were assayed in the IL-5R binding inhibition assay (Example 2). None of the Fabs inhibited binding of iodinated IL-5 to the IL-5Rα by more than 35%.

H. *Conversion of Neutralizing mAb to a FAB:*

The Fd and κ cDNAs of mAb (2B6) were isolated by PCR using the conditions described above. The gel-purified fragments were subcloned into the pMKFabGene3 vector which had been modified to include the hexa-His sequence 3' of the gene III cDNA, resulting in the plasmid pMKFabGene3H. A functional, IL-5 binding Fab clone containing the 2B6 heavy and light chains was identified by a colony lift assay. Upon removal gene III via Nhe I/SpeI I digestion and self-ligation the heavy chain was fused in frame to the hexa-His, allowing purification as described above. In a dose dependent manner, this Fab inhibited receptor binding with an IC_{50} of approximately 7.5 ug/ml, similar to that of the parent mAb, murine 2B6.

I. *Construction and Screening of Chain-Shuffled Library:*

The cDNA encoding the Fd of the neutralizing mAb 2B6 was subcloned as an XhoI/SpeI fragment into pMKFabGene3H which contained a SstI/XbaI fragment in lieu of a light chain cDNA. This phagemid was digested with SstI and XbaI and ligated with the SstI/XbaI digested light chain PCR product derived from the IL-5 immunized mice (described above). The library titer following ligation was 4 X 10^5 CFU. Biopanning, and colony lift assay was performed as described above for the combinatorial library.

The library was constructed by pairing the cDNA encoding the Fd of the neutralizing mAb 2B6 with the same light chain repertoire, recovered from the IL-5 immunized mice, used to generate the combinatorial library. This chain shuffled library was subjected to 4 rounds of biopanning vs immobilized IL-5 and the resultant colonies were assayed for IL-5 reactivity using the colony lift assay. Positive colonies, which bound iodinated IL-5, were further assayed by ELISA and the IL-5Rα binding assay. Two of the Fabs, 2 & 15, recovered from the chain shuffled library blocked binding of IL-5 to the IL-5Rα and inhibited IL-5 dependent proliferation in the B13 assay. The sequences of these 2 Vks were similar to the sequence of the 2B6 Vk, the original light chain partner for the 2B6 V_H. The light chain sequences for Fab 2 & 15 are SEQ ID NOs: 45 and 46, respectively. For Fab 2, CDRs 1-3 are SEQ ID NOs: 10, 11 and 47, respectively. For Fab 15, CDRs 1-3 are SEQ ID NOs: 10, 11 and 48, respectively.

All antibody amino acid sequences listed below in Examples 4 and 5 use the KABAT numbering system which allows variability in CDR and framework lengths. That is, key amino acids are always assigned the same number regardless of the actual

number of amino acids preceding them. For example, the cysteine preceding CDR1 of all light chains is always KABAT position 23 and the tryptophan residue following CDR1 is always KABAT position 35 even though CDR1 may contain up to 17 amino acids.

Example 4 - Humanized Antibody

One humanized antibody was designed to contain murine CDRs within a human antibody framework. This humanized version of the IL-5 specific mouse antibody 2B6, was prepared by performing the following manipulations.

A. *Gene Cloning*:

mRNA was isolated from each of the respective 2B6, 2F2 and 2E3 hybridoma cell lines (see Example 1) with a kit obtained from Boehringer Mannheim (Indianapolis, IN) and then reverse transcribed using the primer $(dT)_{15}$ supplied with a cDNA kit (Boehringer Mannheim) to make cDNA. PCR primers specific for mouse immunoglobulin were used to amplify DNA coding for domains extending from amino acid #9 (KABAT numbering system) of the heavy chain variable region to the hinge region and from amino acid #9 (KABAT numbering system) of the light chain variable region to the end of the constant region. Several clones of each antibody chain were obtained by independent PCR reactions.

The mouse gamma 1 hinge region primer used is [SEQ ID NO: 22]:

5' GTACATATGCAAGGCTTACAACCACAATC 3'.

The mouse gamma 2a hinge region primer used is [SEQ ID NO: 23]:

5' GGACAGGGCTTACTAGTGGGCCCTCTGGGCTC 3'

The mouse heavy chain variable region primer used is [SEQ ID NO: 24]:

5' AGGT(C or G)(C or A)A(G or A)CT(G or T)TCTCGAGTC(T or A)GG 3'

The mouse kappa chain constant region primer used is [SEQ ID NO: 25]:

5' CTAACACTCATTCCTGTTGAAGCTCTTGACAATGGG 3'

The mouse light chain variable region primer is [SEQ ID NO: 26]:

5' CCAGATGTGAGCTCGTGATGACCCAGACTCCA 3'

The PCR fragments were cloned into plasmids pGEM7f+ (Promega) that were then transformed into *E. coli* DH5a (Bethesda Research Labs).

B. *DNA Sequencing*:

The heavy and light chain murine cDNA clones from Part A above were sequenced. The results of sequencing of the variable regions of these clones are shown in SEQ ID NOs: 1-6 (Fig. 1-6). Each clone contained amino acids known to

be conserved among mouse heavy chain variable regions or light chain variable regions. The CDR amino acid sequences are listed below.

The CDR regions for the 2B6 heavy chain are SEQ ID NOs: 7, 8 and 9. See Fig. 7. These sequences are encoded by SEQ ID NO:1. The CDR regions for the light chain are SEQ ID NOs: 10, 11 and 12. See Fig. 7. These sequences are encoded by SEQ ID NO:2.

The CDR regions for the 2F2 heavy chain are SEQ ID NOs: 7, 8 and 9. See Fig. 7. These sequences are encoded by SEQ ID NO:3. The CDR regions for the light chain are SEQ ID NOs: 10, 11 and 13. See Fig. 7. These sequences are encoded by SEQ ID NO:4.

The CDR regions for the 2E3 heavy chain are SEQ ID NOs: 7, 8 and 14. See Fig. 7. These sequences are encoded by SEQ ID NO:5. The CDR regions for the light chain are SEQ ID NOs: 10, 11 and 13. See Fig. 7. These sequences are encoded by SEQ ID NO:6.

C. *Selection of Human Frameworks*:

Following the cloning of 2B6, the amino acid sequences of the variable region heavy and light chains (Figs. 1 and 2) (SEQ ID NOs: 15 and 16, respectively) were compared with the known murine immunoglobulin sequences in the KABAT and SWISS-PROT (Nuc. Acids Res., 20:2019-2022 (1992)) protein sequence databases in order to assign amino acids to the N-terminal residues. The 2B6 heavy and light chain variable region deduced amino acid sequences were then compared with the human immunoglobulin protein sequence databases in order to identify a human framework for both the heavy and light chains which would most closely match the murine sequence. In addition, the heavy and light chains were evaluated with a positional database generated from structural models of the Fab domain to assess potential conflicts due to amino acids which might influence CDR presentation. Conflicts were resolved during synthesis of the humanized variable region frameworks by substitution of the corresponding mouse amino acid at that location.

The heavy chain framework regions of an antibody obtained from a human myeloma immunoglobulin (COR) was used (E. M. Press and N. M. Hogg, Biochem. J., 117:641-660 (1970)). The human heavy chain framework amino acid sequence was found to be approximately 66% homologous to the 2B6 framework.

For a suitable light chain variable region framework, the light chain variable framework sequence of the Bence-Jones protein, (LEN) (Schneider et al., Hoppe-Seyler's Z. Physiol. Chem., 356:507-557 (1975)), was used. The human

light chain framework regions were approximately 82% homologous to the murine 2B6 light chain framework regions, at the amino acid level.

The selected human frameworks were back translated to provide a DNA sequence.

D. *Construction of Humanized MAb Genes*:

Given the 2B6 heavy chain CDRs [Fig. 7 and SEQ ID NOs: 1-2] and the framework sequences of the human antibodies, a synthetic heavy chain variable region was made [SEQ ID NO: 18]. This was made using four synthetic oligonucleotides [SEQ ID NOs:27 and 28] [SEQ ID NOs: 29 and 30] which, when joined, coded for amino acids #21-#106 (KABAT numeration). The oligonucleotides were then ligated into the HpaI-KpnI restriction sites of a pUC18 based plasmid containing sequences derived from another humanized heavy chain based on the COR framework (*supra*). This plasmid provides a signal sequence [SEQ ID NO: 17] and the remaining variable region sequence. Any errors in the mapped sequence were corrected by PCR with mutagenic primers or by the addition of synthetic linkers into existing restriction sites.

The signal sequence and humanized heavy chain variable region were excised from the pUC based plasmid as a EcoRI-ApaI fragment and ligated into the expression vector pCD that contained an IgG_1 human constant region. The synthetic heavy chain variable region nucleotide and amino acid sequences are provided in Fig. 8 [SEQ ID NOs:18 and 19]. The human framework residues are amino acids 1-30, 36-49, 66-97 and 109-119 of SEQ ID NO: 19. The amino acid sequences of the CDRs are identical to the murine 2B6 CDRs. The resulting expression vector, pCDIL5HZHC1.0, is shown in Fig. 10.

Given the 2B6 light chain CDRs [Fig. 7 and SEQ ID NOs: 10, 11 and 12] and the framework sequence of the human antibody, a synthetic light chain variable region was made [SEQ ID NO: 20]. Four synthetic oligonucleotides coding for amino acids #27-#58 (KABAT numeration)[SEQ ID NOs:31 and 32] and amino acids #80-#109 [SEQ ID NOs:33 and 34] of the humanized V_L with SacI-KpnI and PstI-HindIII ends respectively, were inserted into a pUC18 based plasmid containing sequences derived from another human light chain framework (B17) (Marsh et al, Nuc. Acids Res., 13:6531-6544 (1985)) which shares a high degree of homology to the LEN framework. This plasmid provides the remaining variable region sequence. Any errors in the mapped sequence and the single amino acid difference between the LEN and B17 frameworks were corrected by PCR with mutagenic primers or by the addition of synthetic linkers into existing restriction sites.

The humanized light chain variable region was isolated from the pUC plasmid as a EcoRV-NarI fragment and ligated into the expression vector pCN that contained a signal sequence [SEQ ID NO: 17] along with a kappa human constant region. The synthetic light chain variable region nucleotide and amino acid sequences are provided in Fig. 9 [SEQ ID NOs:20 and 21]. The human framework residues are amino acids 1-23, 41-55, 63-94 and 104-113 of SEQ ID NO: 21. The amino acid sequences of the CDRs are identical to the murine 2B6 CDRs. However, the coding sequences for these CDRs differ from the murine 2B6 coding sequences to allow creation of restriction enzyme sites. One of the resulting expression vectors, pCNIL5HZLC1.0, is shown in Fig. 11. These synthetic variable light and/or heavy chain sequences are employed in the construction of a humanized antibody.

E. *Expression of Humanized MAb*:

The humanized heavy chain, derived from an IgG_1 isotype, utilizes a synthetic heavy chain variable region as provided in SEQ ID NO:19. This synthetic V_H containing the 2B6 heavy chain CDRs was designed and synthesized as described above.

The humanized light chain, a human kappa chain, utilizes a synthetic light chain variable region as provided in SEQ ID NO: 21. This synthetic V_L containing the 2B6 light chain CDRs was designed and synthesized as described above. The DNA fragments coding for the humanized variable regions were inserted into pUC19-based mammalian cell expression plasmids that utilize a signal sequence and contain CMV promoters and the human heavy chain or human light chain constant regions of the chimera produced in Example 5 below, by conventional methods (Maniatis *et al.*, cited above) to yield the plasmids pCDIL5HZHC1.0 (heavy chain) [SEQ ID NO: 49, see also FIG. 10] and pCNIL5HZLC1.0 (light chain) [SEQ ID NO: 50, see also FIG. 11]. The plasmids were co-transfected into COS cells and supernatants assayed after three and five days, respectively, by the ELISA described in Example 5 for the presence of human antibody.

The above example describes the preparation of an exemplary engineered antibody. Similar procedures may be followed for the development of other engineered antibodies, using other anti-IL-5 antibodies (e.g., 2F2, 2E3, 4A6, 5D3, 24G9, etc.) developed by conventional means.

F. *Purification*:

Purification of CHO expressed chimeric and humanized 2B6 can be achieved by conventional protein A (or G) affinity chromatography followed by ion exchange and molecular sieve chromatography. Similar processes have been successfully

employed for the purification to >95% purity of other mAbs (e.g., to respiratory syncytial virus, interleukin-4 and malaria circumsporozoite antigens).

G. *Additional Humanized mAbs and Expression Plasmids*:

Given the plasmid pCDIL5HZHC1.0 [SEQ ID NO: 49] the expression plasmid pCDIL5HZHC1.1 was made that substitutes an Asparagine for Threonine at framework position 73. This was done by ligating a synthetic linker with EcoRV and XhoI ends [SEQ ID NO: 51 and SEQ ID NO: 52] into identically digested pCDIL5HZHC1.0. Similarly, the expression plasmid pCDIL5HZHC1.2 substitutes an Isoleucine for Valine at framework position 37. This was accomplished by ligating a synthetic linker with HpaI and XbaI ends [SEQ ID NO: 53 and SEQ ID NO: 54] into identically digested pCDIL5HZHC1.0. The expression plasmid pCDIL5HZHC1.3 was also made by ligating a synthetic linker with HpaI and XbaI ends [SEQ ID NO: 53 and SEQ ID NO: 54] into identically digested pCDIL5HZHC1.1.

Given the pUC18 based plasmid described previously which contains DNA sequences of four synthetic oligonucleotides [SEQ ID NOs: 31, 32, 33 and 34], a humanized light chain variable region was made where framework position #15 is changed from a Leucine to Alanine. This plasmid was digested with NheI and SacI restriction endonucleases and a synthetic linker [SEQ ID NOs: 55 and 56] was inserted. An EcoRV-NarI fragment was then isolated and ligated into the identically digested expression vector pCNIL5HZLC1.0 to create pCNIL5HZLC1.1.

A synthetic variable region was made using the heavy chain framework regions obtained from immunoglobulin (NEW) (Saul et al, J. Biol. Chem. 253:585-597(1978)) and the 2B6 heavy chain CDRs [Fig. 7 and SEQ ID NOs: 1-2]. Framework amino acids which might influence CDR presentation were identified and substitutions made using methods described previously. Four overlapping synthetic oligonucleotides were generated [SEQ ID NOs: 57, 58, 59 and 60] which, when annealed and extended, code for amino acids representing a signal sequence [SEQ ID NO: 17] and a heavy chain variable region. This synthetic gene was then amplified using PCR primers [SEQ ID NOs: 63 and 64] and ligated as a BstXI-HindIII restriction fragment into a pUC18 based plasmid containing sequences derived from another humanized heavy chain based on the COR framework. A phenylalanine to tyrosine framework substitution was made at amino acid position 91 (Kabat numbering system) (equivalent to position 94 of Figure 12) by inserting a synthetic oligonucleotide linker [SEQ ID NOs: 75 and 76] into SacII and KpnI restriction sites. The resulting heavy chain variable region [Fig. 12 and SEQ ID NOs: 61, 62] is referred to as the NEWM humanized heavy chain.

Any errors in the mapped sequence were corrected by PCR with mutagenic primers or by the addition of synthetic linkers into existing restriction sites. The signal sequence and humanized heavy chain variable region were excised from the pUC based plasmid as a EcoRI-ApaI fragment and ligated into the expression vector pCD that contained a human IgG_1 constant region to create the plasmid pCDIL5NEWM. The amino acid sequences of the CDRs are identical to the murine 2B6 heavy chain CDRs.

A synthetic variable region was made using the light chain framework regions obtained from immunoglobulin (REI) (Palm et al, Hoppe-Seyler's Z. Physiol. Chem. 356:167-191(1975)) and the 2B6 light chain CDRs [Fig. 7 and SEQ ID NOs: 10, 11 and 12]. Framework amino acids which might influence CDR presentation were identified and substitutions made using methods described previously. Four overlapping synthetic oligonucleotides were generated [SEQ ID NOs: 65, 66, 67 and 68] which, when annealed and extended, code for amino acids representing a light chain variable region [Fig. 13 and SEQ ID NOs: 69, 70] referred to as the REI humanized light chain. This synthetic gene was then amplified using PCR primers [SEQ ID NOs: 71 and 72] and ligated as an EcoRI-HindIII restriction fragment into pGEM-7Zf(+) (Promega Corporation, Madison, WI).

Any errors in the mapped sequence were corrected by PCR with mutagenic primers or by the addition of synthetic linkers into existing restriction sites. The humanized light chain variable region was excised from the pGEM-7Zf(+) based plasmid as an EcoRV-NarI fragment and ligated into the expression vector pCN that contained a signal sequence [SEQ ID NO: 17] along with a human Kappa constant region to create the plasmid pCNIL5REI. The amino acid sequences of the CDRs are identical to the murine 2B6 light chain CDRs. However, the coding sequences for these CDRs differ from the murine 2B6 coding sequences to allow creation of restriction enzyme sites. These synthetic variable light and/or heavy chain sequences are employed in the construction of a humanized antibody.

Given the pGEM-7Zf(+) based plasmid described above, a humanized light chain variable region can be made where framework position #15 is changed from a Valine to Alanine. This plasmid may be digested with NheI and SacI restriction endonucleases and a synthetic linker [SEQ ID NOs: 73 and 74] is inserted. An EcoRV-NarI fragment may then be isolated and ligated into the identically digested expression vector pCNIL5HZREI to create the plasmid $pCNIL5REI_{V15A}$.

Example 5 - Construction of a Chimeric Antibody

DNA coding for amino acids #9-#104 (KABAT numeration) of the murine mAb 2B6 heavy chain variable region was isolated as a AvaII-StyI restriction fragment from a pGEM7Zf+ based PCR clone of cDNA generated from the 2B6 hybridoma cell line (see Example 4). The flanking heavy chain variable region sequences and a signal sequence [SEQ ID NO: 17] were provided by combining this fragment along with four small synthetic oligomer linkers [SEQ ID NOs: 35 and 36] [SEQ ID NOs: 37 and 38] into a pUC18 based plasmid digested with BstX1-HindIII. A consensus of N-terminal amino acids deduced from closely related murine heavy chains were assigned for the first eight V_H residues and are coded within SEQ ID NOs: 35 and 36. The deduced amino acid sequence of the heavy chain was verified by the sequencing of the first 15 N-terminal amino acids of the 2B6 heavy chain.

An EcoRI-ApaI fragment containing sequence for signal and V_H regions was isolated and ligated into plasmid pCD that already encodes the human IgG1 constant region.

DNA coding for amino acids #12-#99 (KABAT nomenclature) of the murine mAb 2B6 light chain variable region was isolated as a DdeI-AvaI restriction fragment from a pGEM7Zf+ based PCR clone of cDNA generated from the 2B6 hybridoma cell line (see Example 4). The flanking light chain variable region sequences were provided by combining this fragment along with four small synthetic oligomer linkers [SEQ ID NOs: 39 and 40] [SEQ ID NOs: 41 and 42] into a pUC18 based plasmid digested with EcoRV-HindIII. A consensus of N-terminal amino acids deduced from closely related murine light chains were assigned for the first eight V_L residues and are coded within SEQ ID NOs: 39 and 40. The deduced amino acid sequence of the light chain was verified by the sequencing of the first 15 N-terminal amino acids of the 2B6 light chain. This variable region was then isolated as a EcoRV-NarI fragment and ligated into the expression vector pCN that already contains the human kappa region and a signal sequence.

Expression of a chimeric antibody was accomplished by co-transfection of the pCD and pCN based plasmids into COS cells. Culture supernatants were collected three and five days later and assayed for immunoglobulin expression by ELISA described as follows: Each step except for the last is followed by PBS washes. Microtiter plates were coated overnight with 100 ng/50 ul/well of a goat antibody specific for the Fc region of human antibodies. The culture supernatants

were added and incubated for 1 hour. Horseradish peroxidase conjugated goat anti-human IgG antibody was then added and allowed to incubate for 1 hour. This was followed by addition of ABTS peroxidase substrate (Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD). After 1 hour incubation, the absorbance at 405 nm was read on a microtiter plate reader (Molecular Devices Corporation, Menlo Park, CA). Expression of the chimeric antibody was detected. In a similar ELISA, the COS cell supernatants, containing the chimeric antibody, bound specifically to microtiter wells coated with human IL-5 protein. This result confirmed that genes coding for an antibody to IL-5 had been synthesized and expressed.

The above example describes the preparation of an exemplary engineered antibody. Similar procedures may be followed for the development of other engineered antibodies, using other anti-IL-5 donor antibodies (e.g., 2F2, 2E3, 4A6, 5D3, 24G9, etc.) developed by conventional means.

Example 6 - Human IL-5/hIL-5 Receptor α-chain/MAb Complex

A. *ELISA*

The following antibodies were evaluated: 24G9 (non-neutralizing), 4A6 (neutralizing) and 2B6 (neutralizing), at concentrations between 2ng/ml - 32ng/ml.

Flat bottomed ELISA plate wells (Wallac) were coated overnight with 100ul Protein-A (5ug/ml) at 4° C. Following aspiration, wells were blocked with 200ul blocking buffer containing 1% BSA and incubated for 60 min at 37° C. Plates were washed 4x and 100ul soluble IL-5Ralpha-Fc (see Johnson et al., (1995) J Biol Chem, 270: 9459-9471) (2.25ug/ml) added to each well and incubated at 37° C for 30 min. Plates were washed 1x and increasing concentrations of recombinant human IL-5 added to each well. Following a 30 min incubation at 37° C, wells were washed once and 100ul antibody (2ug/ml) added and incubated for 60 min at 37° C. Plates were washed 4x and 100ul biotin-labelled goat anti-mouse Ig or goat anti-rat Ig (Sigma) added to each well. Plates were incubated for 60 min at 37° C and washed 4x. Europium-labelled streptavidin (Wallac) was diluted to 1:1000 in europium buffer (Wallac) and 100ul volumes added to each well. Plates were incubated for 30 min at 37° C and washed 6x. Enhancer solution (Wallac) was added to each well and plates read using a 1234 Delphia research fluorometer.

There was a dose-dependent increase in binding of mAbs 24G9 and 4A6 to the IL-5/IL-5 receptor complex. No such increase was seen using mAb 2B6. MAb 2B6 inhibits binding of IL-5 to the IL-5Ralpha chain. In contrast neither 24G9 or 4A6 inhibited binding of IL-5 to the IL-5Ralpha chain. See Table II.

TABLE II

Binding to hIL-5/IL-5 Receptor α-chain Complex (Counts)

Concentration (ng/ml/mAb)	24G9	2B6	4A6
0	4975	2767	6733
2	6338	2939	8227
8	10521	3196	13957
32	33911	4977	33143

B. *Optical Biosensor*

(1) MAb 4A6 was immobilized onto a BIAcore chip (see Example 2D). hIL-5 (25ul) was passed onto the 4A6 surface at a flow rate of 5ul/min. IL-5 receptor α-chain (@15, 30, 60, 120nM, in 25ul) was then injected. The kinetics of IL-5 receptor α-chain (Ra) in binding to 4A6/IL-5 complex can be calculated as: Kon=$6.3x10^5$(/M/s); Koff=$2.3x10^{-3}$ (/s). The kinetics for IL-5-IL-5Pα interaction is: Kon=$7.5x10^5$(/M/s); Koff=$2.8x10^{-3}$ (/s). Thus, mAb 4A6 has no significant effect on the interaction between IL-5 and IL-5 receptor α-chain.

(2) hIL-5 was immobilized on a BIAcore chip. MAb 4A6 (25ul, 32ug/ml) was injected onto the surface, followed by injection of IL-5 receptor α-chain (20ul, 90nM). As a control, the same amount of IL-5 receptor α-chain was injected directly onto the IL-5 surface. Pre-binding of mAb 4A6 to hIL5 did not block binding of IL-5 receptor α-chain to hIL-5.

(3) Protein A was immobilized on a BIAcore chip. IL-5Ra-Fc (30ul, 20ug/ml) was injected onto the protein A surface. IL-5 (25ul, 80nM) was then captured by IL-5Rα-Fc, followed by binding of mAb 24G9 (25ul, 32ug/ml). MAb 24G9 bound to the hIL-5/IL-5Rα complex.

(4) MAb 24G9 was immobilized on a BIAcore chip. IL-5 was captured to the 24G9 surface, followed by injections of different mAbs. Mabs 2B6, TRFK5 and CMX5-2 bind to the IL-5/24G9 complex, while binding of mAb 4A6 to IL-5 was blocked. This indicates that 4A6 and 24G9 share binding epitopes.

C. *Sedimentation Velocity*

A three component mixture of hIL-5, mAb 4A6, and a soluble IL-5 receptor α-chain was analyzed by sedimentation velocity after 14 hours (20°C). A

complex corresponding to the same size as a soluble IL-5 receptor α-chain/hIL-5/mAb complex was observed.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Cook, Richard M.
Appelbaum, Edward R.

(ii) TITLE OF INVENTION: Improved Method for Treatment and Diagnosis of IL-5 Mediated Disorders

(iii) NUMBER OF SEQUENCES: 76

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: SmithKline Beecham Corp./Corporate
(B) STREET: P.O. Box 1539-UW2220
(C) CITY: King of Prussia
(D) STATE: Pennsylvania
(E) COUNTRY: USA
(F) ZIP: 19406-0939

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/470110
(B) FILING DATE: 06-JUN-1995

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 08/467420
(B) FILING DATE: 06-JUN-1995

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/363131
(B) FILING DATE: 23-DEC-1994

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Sutton, Jeffrey A.
(B) REGISTRATION NUMBER: 34,028
(C) REFERENCE/DOCKET NUMBER: P50282-2

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 610-270-5024
(B) TELEFAX: 610-270-5090

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 334 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..334
(D) OTHER INFORMATION: /note= "First base corresponds to Kabat position 24"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ACCTGGCCTG GTGGCGCCCT CACAGAGCCT GTCCATCACT TGCACTGTCT CTGGGTTTTC 60

ATTAACCAGC TATAGTGTAC ACTGGGTTCG CCAGCCTCCA GGAAAGGGTC TGGAGTGGCT 120

GGGAGTAATA TGGGCTAGTG GAGGCACAGA TTATAATTCG GCTCTCATGT CCAGACTGAG 180

CATCAGCAAA GACAACTCCA AGAGCCAAGT TTTCTTAAAA CTGAACAGTC TGCAAACTGA 240

TGACACAGCC ATGTACTACT GTGCCAGAGA TCCCCCTTCT TCCTTACTAC GGCTTGACTA 300

CTGGGGCCAA GGCACCACTC TCACAGTCTC CTCA 334

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 315 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..315
(D) OTHER INFORMATION: /note= "First base corresponds to Kabat position 25"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TCCTCCCTGA GTGTGTCAGC AGGAGAGAAG GTCACTATGA GCTGCAAGTC CAGTCAGAGT 60

CTGTTAAACA GTGGAAATCA AAAGAACTAC TTGGCCTGGT ACCAGCAGAA ACCAGGGCAG 120

CCTCCTAAAC TTTTGATCTA CGGGGCATCC ACTAGGGAAT CTGGGGTCCC TGATCGCTTC 180

ACAGGCAGTG GATCTGGAAC CGATTTCACT CTTTCCATCA GCAGTGTGCA GGCTGAAGAC 240

CTGGCAGTTT ATTACTGTCA GAATGTTCAT AGTTTTCCAT TCACGTTCGG CTCGGGGACA 300

GAGTTGGAAA TAAAA 315

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 334 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..334
(D) OTHER INFORMATION: /note= "First base corresponds to Kabat position 24"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ACCTGGCCTG GTGGCGCCCT CACAGAGCCT GTCCATCACT TGCACTGTCT CTGGGTTTTC 60

ATTAACCAGT TATAGTGTAC ACTGGGTTCG CCAGCCTCCA GGAAAGGGTC TGGAGTGGCT 120

GGGAGTAATA TGGGCTAGTG GAGGCACAGA TTATAATTCG GCTCTCATGT CCAGACTGAG 180

CATCAGCAAA GACAACTCCA AGAGCCAAGT TTTCTTAAAA CTGAACAGTC TGCGAACTGA 240

TGACACAGCC ATGTACTACT GTGCCAGAGA TCCCCCTTCT TCCTTACTAC GGCTTGACTA 300

CTGGGGCCAA GGCACCACTC TCACAGTCTC CTCA 334

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 315 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..315
(D) OTHER INFORMATION: /note= "First base corresponds to Kabat 25"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TCCTCCCTGA GTGTGTCAGC AGGAGAGAAG GTCACTATGA GCTGCAAGTC CAGTCAGAGT 60

CTATTAAACA GTGGAAATCA AAAGAACTAC TTGGCCTGGT ACCAACAGAA ACCAGGGCAG 120

CCTCCTAAAC TTTTGATCTA CGGGGCATCC ACTAGGGAAT CTGGGGTCCC TGATCGCTTC 180

ACAGGCAGTG GATCTGGAAC CGATTTCACT CTTACCATCA GCAGTGTGCA GGCTGAAGAC 240

CTGGCAGTTT ATTACTGTCA GAATGATCAT AGTTTTCCAT TCACGTTCGG CTCGGGGACA 300

GAGTTGGAAA TAAAA 315

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 334 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..334
(D) OTHER INFORMATION: /note= "First base corresponds to Kabat position 24"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5.

ACCTGGCCTG GTGGCGCCCT CACAGAGCCT GTCCATCACT TGCACTGTCT CTGGGTTTTC 60

ATTAACCAGC TATAGTGTAC ACTGGGTTCG CCAGCCTCCA GGAAAGGGTC TGGAGTGGCT 120

GGGAGTAATC TGGGCTAGTG GAGGCACAGA TTATAATTCG GCTCTCATGT CCAGACTGAG 180

CATCAGCAAA GACAACTCCA AGAGCCAAGT TTTCTTAAAA CTGAACAGTC TGCAAACTGA 240

TGACGCAGCC ATGTACTACT GTGCCAGAGA TCCCCCTTTT TCCTTACTAC GGCTTGACTT 300

CTGGGGCCAA GGCACCACTC TCACAGTCTC CTCA 334

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 315 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..315
(D) OTHER INFORMATION: /note= "First base corresponds to Kabat position 25"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TCCTCTCTGA GTGTGTCAGC AGGAGAGAAG GTCACTATGA GCTGCAAGTC CAGTCAGAGT 60

CTGTTAAACA GTGGAAATCA AAAAAACTAC TTGGCCTGGT ACCAGCAGAA ACCAGGGCAG 120

CCTCCTAAAC TTTTGATCTA CGGGGCATCC ACTAGGGAAT CTGGGGTCCC TGATCGCTTC 180

ACAGGCAGTG GATCTGGAAC CGATTTCACT CTTACCATCA GCAGTGTGCA GGCTGAAGAC 240

CTGGCAGTTT ATTACTGTCA GAATGATCAT AGTTTTCCAT TCACGTTCGG CTCGGGGACA 300

GAGTTGGAAA TAAAA 315

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Ser Tyr Ser Val His
1 5

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Val Ile Trp Ala Ser Gly Gly Thr Asp Tyr Asn Ser Ala Leu Met Ser
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Asp Pro Pro Ser Ser Leu Leu Arg Leu Asp Tyr
1 5 10

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Lys Ser Ser Gln Ser Leu Leu Asn Ser Gly Asn Gln Lys Asn Tyr Leu
1 5 10 15

Ala

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Gly Ala Ser Thr Arg Glu Ser
1 5

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Gln Asn Val His Ser Phe Pro Phe Thr
1 5

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Gln Asn Asp His Ser Phe Pro Phe Thr
1 5

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Asp Pro Pro Phe Ser Leu Leu Arg Leu Asp Phe
1 5 10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Ala Pro Ser Gln
1 5 10 15

Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Ser Tyr
20 25 30

Ser Val His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45

Gly Val Ile Trp Ala Ser Gly Gly Thr Asp Tyr Asn Ser Ala Leu Met
50 55 60

Ser Arg Leu Ser Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Phe Leu
65 70 75 80

Lys Leu Asn Ser Leu Gln Thr Asp Asp Thr Ala Met Tyr Tyr Cys Ala
85 90 95

Arg Asp Pro Pro Ser Ser Leu Leu Arg Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Thr Leu Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 113 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Asp Ile Val Met Thr Gln Ser Pro Ser Ser Leu Ser Val Ser Ala Gly
1 5 10 15

Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser
20 25 30

Gly Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45

Pro Pro Lys Leu Leu Ile Tyr Gly Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Ser
65 70 75 80

Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Asn
85 90 95

Val His Ser Phe Pro Phe Thr Phe Gly Ser Gly Thr Glu Leu Glu Ile
100 105 110

Lys

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

ATGGTGTTGC AGACCCAGGT CTTCATTTCT CTGTTGCTCT GGATCTCTGG TGCCTACGGG 60

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 357 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CAGGTTACCC TGCGTGAATC CGGTCCGGCA CTAGTTAAAC CGACCCAGAC CCTGACGTTA 60

ACCTGCACCG TCTCCGGTTT CTCCCTGACG AGCTATAGTG TACACTGGGT CCGTCAGCCG 120

CCGGGTAAAG GTCTAGAATG GCTGGGTGTA ATATGGGCTA GTGGAGGCAC AGATTATAAT 180

TCGGCTCTCA TGTCCCGTCT GTCGATATCC AAAGACACCT CCCGTAACCA GGTTGTTCTG 240

ACCATGACTA ACATGGACCC GGTTGACACC GCTACCTACT ACTGCGCTCG AGATCCCCCT 300

TCTTCCTTAC TACGGCTTGA CTACTGGGGT CGTGGTACCC CAGTTACCGT GAGCTCA 357

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 119 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Gln	Val	Thr	Leu	Arg	Glu	Ser	Gly	Pro	Ala	Leu	Val	Lys	Pro	Thr	Gln
1				5				10						15	

Thr	Leu	Thr	Leu	Thr	Cys	Thr	Val	Ser	Gly	Phe	Ser	Leu	Thr	Ser	Tyr
			20					25					30		

Ser	Val	His	Trp	Val	Arg	Gln	Pro	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Leu
		35					40					45			

Gly	Val	Ile	Trp	Ala	Ser	Gly	Gly	Thr	Asp	Tyr	Asn	Ser	Ala	Leu	Met
	50					55					60				

Ser Arg Leu Ser Ile Ser Lys Asp Thr Ser Arg Asn Gln Val Val Leu
65 70 75 80

Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
85 90 95

Arg Asp Pro Pro Ser Ser Leu Leu Arg Leu Asp Tyr Trp Gly Arg Gly
100 105 110

Thr Pro Val Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 339 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GATATCGTGA TGACCCAGTC TCCAGACTCG CTAGCTGTGT CTCTGGGCGA GAGGGCCACC 60

ATCAACTGCA AGAGCTCTCA GAGTCTGTTA AACAGTGGAA ATCAAAAGAA CTACTTGGCC 120

TGGTATCAGC AGAAACCCGG GCAGCCTCCT AAGTTGCTCA TTTACGGGGC GTCGACTAGG 180

GAATCTGGGG TACCTGACCG ATTCAGTGGC AGCGGGTCTG GGACAGATTT CACTCTCACC 240

ATCAGCAGCC TGCAGGCTGA AGATGTGGCA GTATACTACT GTCAGAATGT TCATAGTTTT 300

CCATTCACGT TCGGCGGAGG GACCAAGTTG GAGATCAAA 339

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 113 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser
20 25 30

Gly Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45

Pro Pro Lys Leu Leu Ile Tyr Gly Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Asn
85 90 95

Val His Ser Phe Pro Phe Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
100 105 110

Lys

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GTACATATGC AAGGCTTACA ACCACAATC 29

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GGACAGGGCT TACTAGTGGG CCCTCTGGGC TC 32

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

AGGTSMARCT RTCTCGAGTC WGG 33

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CTAACACTCA TTCCTGTTGA AGCTCTTGAC AATGGG 36

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 32 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CCAGATGTGA GCTCGTGATG ACCCAGACTC CA 32

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 140 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

AACCTGCACC GTCTCCGGTT TCTCCCTGAC GAGCTATAGT GTACACTGGG TCCGTCAGCC 60

GCCGGGTAAA GGTCTAGAAT GGCTGGGTGT AATATGGGCT AGTGGAGGCA CAGATTATAA 120

TTCGGCTCTC ATGTCCCGTC 140

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 149 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

ATATCGACAG ACGGGACATG AGAGCCGAAT TATAATCTGT GCCTCCACTA GCCCATATTA 60

CACCCAGCCA TTCTAGACCT TTACCCGGCG GCTGACGGAC CCAGTGTACA CTATAGCTCG 120

TCAGGGAGAA ACCGGAGACG GTGCAGGTT 149

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 139 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

TGTCGATATC CAAAGACACC TCCCGTAACC AGGTTGTTCT GACCATGACT AACATGGACC 60

CGGTTGACAC CGCTACCTAC TACTGCGCTC GAGATCCCCC TTCTTCCTTA CTACGGCTTG 120

ACTACTGGGG TCGTGGTAC 139

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 126 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

CACGACCCCA GTAGTCAAGC CGTAGTAAGG AAGAAGGGGG ATCTCGAGCG CAGTAGTAGG 60

TAGCGGTGTC AACCGGGTCC ATGTTAGTCA TGGTCAGAAC AACCTGGTTA CGGGAGGTGT 120

CTTTGG 126

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 117 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

CTCAGAGTCT GTTAAACAGT GGAAATCAAA AGAACTACTT GGCCTGGTAT CAGCAGAAAC 60

CCGGGCAGCC TCCTAAGTTG CTCATTTACG GGGCGTCGAC TAGGGAATCT GGGGTAC 117

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 117 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

CCCAGATTCC CTAGTCGACG CCCCGTAAAT GAGCAACTTA GGAGGCTGCC CGGGTTTCTC 60

CTGATACCAG GCCAAGTAGT TCTTTTGATT TCCACTGTTT AACAGACTCT GAGAGCT 117

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 102 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GCTGAAGATG TGGCAGTATA CTACTGTCAG AATGTTCATA GTTTTCCATT CACGTTCGGC 60

GGAGGGACCA AGTTGGAGAT CAAACGTACT GTGGCGGCGC CA 102

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 111 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

AGCTTGGCGC CGCCACAGTA CGTTTGATCT CCAACTTGGT CCCTCCGCCG AACGTGAATG 60

GAAAACTATG AACATTCTGA CAGTAGTATA CTGCCACATC TTCAGCCTGC A 111

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 82 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

ATGGTGTTGC AGACCCAGGT CTTCATTTCT CTGTTGCTCT GGATCTCTGG TGCCTACGGG 60

CAGGTTCAAC TGAAAGAGTC AG 82

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 89 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GTCCTGACTC TTTCAGTTGA ACCTGCCCGT AGGCACCAGA GATCCAGAGC AACAGAGAAA 60

TGAAGACCTG GGTCTGCAAC ACCATGTTG 89

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 45 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

CAAGGCACCA CTCTCACAGT CTCCTCAGCT AGTACGAAGG GCCCA 45

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 43 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

AGCTTGGGCC CTTCGTACTA GCTGAGGAGA CTGTGAGTGG TGC 43

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

ATCGTGATGA CCCAGTCTCC ATCCTCCC 28

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

TCAGGGAGGA TGGAGACTGC GTCATCACGA T 31

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

TCGGGGGACA GAGTTGGAAA TAAAACGTAC TGTGGCGGCG CCA 43

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

AGCTTGGCGC CGCCACAGTA CGTTTTATTT CCAACTCTGT CC 42

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

CTAGCCACCA CCACCACCAC CACTAA 26

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

CTAGTTAGTC GTGGTGGTGG TGGTGG 26

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 113 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Glu Leu Val Met Thr Gln Ser Pro Ser Ser Leu Ser Val Ser Ala Gly
1 5 10 15

Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser
20 25 30

Gly Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45

Pro Pro Lys Leu Leu Ile Tyr Gly Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Asn
85 90 95

Asp His Ser Tyr Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile
100 105 110

Lys

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 113 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Glu Leu Val Met Thr Gln Ser Pro Ser Ser Leu Ser Val Ser Ala Gly
1 5 10 15

Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser
20 25 30

Gly Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45

Pro Pro Lys Leu Leu Ile Tyr Gly Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Asn
85 90 95

Asp Tyr Ser Tyr Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile
100 105 110

Lys

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Gln Asn Asp His Ser Tyr Pro Phe Thr
1 5

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Gln Asn Asp Tyr Ser Tyr Pro Phe Thr
1 5

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6285 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

GACGTCGCGG CCGCTCTAGG CCTCCAAAAA AGCCTCCTCA CTACTTCTGG AATAGCTCAG 60

AGGCCGAGGC GGCCTCGGCC TCTGCATAAA TAAAAAAAAT TAGTCAGCCA TGCATGGGGC 120

GGAGAATGGG CGGAACTGGG CGGAGTTAGG GGCGGGATGG GCGGAGTTAG GGGCGGGACT 180

ATGGTTGCTG ACTAATTGAG ATGCATGCTT TGCATACTTC TGCCTGCTGG GGAGCCTGGG 240

GACTTTCCAC ACCTGGTTGC TGACTAATTG AGATGCATGC TTTGCATACT TCTGCCTGCT 300

GGGGAGCCTG GGGACTTTCC ACACCCTAAC TGACACACAT TCCACAGAAT TAATTCCCGG 360

GGATCGATCC GTCGACGTAC GACTAGTTAT TAATAGTAAT CAATTACGGG GTCATTAGTT 420

CATAGCCCAT ATATGGAGTT CCGCGTTACA TAACTTACGG TAAATGGCCC GCCTGGCTGA 480

CCGCCCAACG ACCCCCGCCC ATTGACGTCA ATAATGACGT ATGTTCCCAT AGTAACGCCA 540

ATAGGGACTT TCCATTGACG TCAATGGGTG GACTATTTAC GGTAAACTGC CCACTTGGCA 600

GTACATCAAG TGTATCATAT GCCAAGTACG CCCCCTATTG ACGTCAATGA CGGTAAATGG 660

CCCGCCTGGC ATTATGCCCA GTACATGACC TTATGGGACT TTCCTACTTG GCAGTACATC 720

TACGTATTAG TCATCGCTAT TACCATGGTG ATGCGGTTTT GGCAGTACAT CAATGGGCGT 780

GGATAGCGGT TTGACTCACG GGGATTTCCA AGTCTCCACC CCATTGACGT CAATGGGAGT 840

TTGTTTTGGC ACCAAAATCA ACGGGACTTT CCAAAATGTC GTAACAACTC CGCCCCATTG 900

ACGCAAATGG GCGGTAGGCG TGTACGGTGG GAGGTCTATA TAAGCAGAGC TGGGTACGTG 960

AACCGTCAGA TCGCCTGGAG ACGCCATCGA ATTCGAGGAC GCCAGCAACA TGGTGTTGCA 1020

GACCCAGGTC TTCATTTCTC TGTTGCTCTG GATCTCTGGT GCCTACGGGC AGGTTACCCT 1080

GCGTGAATCC GGTCCGGCAC TAGTTAAACC GACCCAGACC CTGACGTTAA CCTGCACCGT 1140

CTCCGGTTTC TCCCTGACGA GCTATAGTGT ACACTGGGTC CGTCAGCCGC CGGGTAAAGG 1200

TCTAGAATGG CTGGGTGTAA TATGGGCTAG TGGAGGCACA GATTATAATT CGGCTCTCAT 1260

GTCCCGTCTG TCGATATCCA AAGACACCTC CCGTAACCAG GTTGTTCTGA CCATGACTAA 1320

CATGGACCCG GTTGACACCG CTACCTACTA CTGCGCTCGA GATCCCCCTT CTTCCTTACT 1380

ACGGCTTGAC TACTGGGGTC GTGGTACCCC AGTTACCGTG AGCTCAGCTA GTACCAAGGC 1440

CCCATCGGTC TTCCCCCTGG CACCCTCCTC CAAGAGCACC TCTGGGGGCA CAGCGGCCCT 1500

GGGCTGCCTG GTCAAGGACT ACTTCCCCGA ACCGGTGACG GTGTCGTGGA ACTCAGGCGC 1560

CCTGACCAGC GGCGTGCACA CCTTCCCGGC TGTCCTACAG TCCTCAGGAC TCTACTCCCT 1620

CAGCAGCGTG GTGACCGTGC CCTCCAGCAG CTTGGGCACC CAGACCTACA TCTGCAACGT 1680

GAATCACAAG CCCAGCAACA CCAAGGTGGA CAAGAGAGTT GAGCCCAAAT CTTGTGACAA 1740

AACTCACACA TGCCCACCGT GCCCAGCACC TGAACTCCTG GGGGGACCGT CAGTCTTCCT 1800

CTTCCCCCCA AAACCCAAGG ACACCCTCAT GATCTCCCGG ACCCCTGAGG TCACATGCGT 1860

GGTGGTGGAC GTGAGCCACG AAGACCCTGA GGTCAAGTTC AACTGGTACG TGGACGGCGT 1920

GGAGGTGCAT AATGCCAAGA CAAAGCCGCG GGAGGAGCAG TACAACAGCA CGTACCGTGT 1980

GGTCAGCGTC CTCACCGTCC TGCACCAGGA CTGGCTGAAT GGCAAGGAGT ACAAGTGCAA 2040

GGTCTCCAAC AAAGCCCTCC CAGCCCCCAT CGAGAAAACC ATCTCCAAAG CCAAAGGGCA 2100

GCCCCGAGAA CCACAGGTGT ACACCCTGCC CCCATCCCGG GAGGAGATGA CCAAGAACCA 2160

GGTCAGCCTG ACCTGCCTGG TCAAAGGCTT CTATCCCAGC GACATCGCCG TGGAGTGGGA 2220

GAGCAATGGG CAGCCGGAGA ACAACTACAA GACCACGCCT CCCGTGCTGG ACTCCGACGG 2280

CTCCTTCTTC CTCTATAGCA AGCTCACCGT GGACAAGAGC AGGTGGCAGC AGGGGAACGT 2340

CTTCTCATGC TCCGTGATGC ATGAGGCTCT GCACAACCAC TACACGCAGA AGAGCCTCTC 2400

CCTGTCTCCG GGTAAGTGAG TGTAGTCTAG ATCTACGTAT GATCAGCCTC GACTGTGCCT 2460

TCTAGTTGCC AGCCATCTGT TGTTTGCCCC TCCCCCGTGC CTTCCTTGAC CCTGGAAGGT 2520

GCCACTCCCA CTGTCCTTTC CTAATAAAAT GAGGAAATTG CATCGCATTG TCTGAGTAGG 2580

TGTCATTCTA TTCTGGGGGG TGGGGTGGGG CAGGACAGCA AGGGGGAGGA TTGGGAAGAC 2640

AATAGCAGGC ATGCTGGGGA TGCGGTGGGC TCTATGGAAC CAGCTGGGGC TCGACAGCGC 2700

TGGATCTCCC GATCCCCAGC TTTGCTTCTC AATTTCTTAT TTGCATAATG AGAAAAAAAG 2760

GAAAATTAAT TTTAACACCA ATTCAGTAGT TGATTGAGCA AATGCGTTGC CAAAAAGGAT 2820

GCTTTAGAGA CAGTGTTCTC TGCACAGATA AGGACAAACA TTATTCAGAG GGAGTACCCA 2880

GAGCTGAGAC TCCTAAGCCA GTGAGTGGCA CAGCATTCTA GGGAGAAATA TGCTTGTCAT 2940

CACCGAAGCC TGATTCCGTA GAGCCACACC TTGGTAAGGG CCAATCTGCT CACACAGGAT 3000

AGAGAGGGCA GGAGCCAGGG CAGAGCATAT AAGGTGAGGT AGGATCAGTT GCTCCTCACA 3060

TTTGCTTCTG ACATAGTTGT GTTGGGAGCT TGGATAGCTT GGACAGCTCA GGGCTGCGAT 3120

TTCGCGCCAA ACTTGACGGC AATCCTAGCG TGAAGGCTGG TAGGATTTTA TCCCCGCTGC 3180

CATCATGGTT CGACCATTGA ACTGCATCGT CGCCGTGTCC CAAAATATGG GGATTGGCAA 3240

GAACGGAGAC CTACCCTGGC CTCCGCTCAG GAACGAGTTC AAGTACTTCC AAAGAATGAC 3300

CACAACCTCT TCAGTGGAAG GTAAACAGAA TCTGGTGATT ATGGGTAGGA AAACCTGGTT 3360

CTCCATTCCT GAGAAGAATC GACCTTTAAA GGACAGAATT AATATAGTTC TCAGTAGAGA 3420

ACTCAAAGAA CCACCACGAG GAGCTCATTT TCTTGCCAAA AGTTTGGATG ATGCCTTAAG 3480

ACTTATTGAA CAACCGGAAT TGGCAAGTAA AGTAGACATG GTTTGGATAG TCGGAGGCAG 3540

TTCTGTTTAC CAGGAAGCCA TGAATCAACC AGGCCACCTT AGACTCTTTG TGACAAGGAT 3600

CATGCAGGAA TTTGAAAGTG ACACGTTTTT CCCAGAAATT GATTTGGGGA AATATAAACT 3660

TCTCCCAGAA TACCCAGGCG TCCTCTCTGA GGTCCAGGAG GAAAAAGGCA TCAAGTATAA 3720

GTTTGAAGTC TACGAGAAGA AAGACTAACA GGAAGATGCT TTCAAGTTCT CTGCTCCCCT 3780

CCTAAAGCTA TGCATTTTTA TAAGACCATG GGACTTTTGC TGGCTTTAGA TCAGCCTCGA 3840

CTGTGCCTTC TAGTTGCCAG CCATCTGTTG TTTGCCCCTC CCCCGTGCCT TCCTTGACCC 3900

TGGAAGGTGC CACTCCCACT GTCCTTTCCT AATAAAATGA GGAAATTGCA TCGCATTGTC 3960

TGAGTAGGTG TCATTCTATT CTGGGGGGTG GGGTGGGGCA GGACAGCAAG GGGGAGGATT 4020

GGGAAGACAA TAGCAGGCAT GCTGGGGATG CGGTGGGCTC TATGGAACCA GCTGGGGCTC 4080

GATCGAGTGT ATGACTGCGG CCGCGATCCC GTCGAGAGCT TGGCGTAATC ATGGTCATAG 4140

CTGTTTCCTG TGTGAAATTG TTATCCGCTC ACAATTCCAC ACAACATACG AGCCGGAAGC 4200

ATAAAGTGTA AAGCCTGGGG TGCCTAATGA GTGAGCTAAC TCACATTAAT TGCGTTGCGC 4260

TCACTGCCCG CTTTCCAGTC GGGAAACCTG TCGTGCCAGC TGCATTAATG AATCGGCCAA 4320

CGCGCGGGGA GAGGCGGTTT GCGTATTGGG CGCTCTTCCG CTTCCTCGCT CACTGACTCG 4380

CTGCGCTCGG TCGTTCGGCT GCGGCGAGCG GTATCAGCTC ACTCAAAGGC GGTAATACGG 4440

TTATCCACAG AATCAGGGGA TAACGCAGGA AAGAACATGT GAGCAAAAGG CCAGCAAAAG 4500

GCCAGGAACC GTAAAAAGGC CGCGTTGCTG GCGTTTTTCC ATAGGCTCCG CCCCCCTGAC 4560

GAGCATCACA AAAATCGACG CTCAAGTCAG AGGTGGCGAA ACCCGACAGG ACTATAAAGA 4620

TACCAGGCGT TTCCCCCTGG AAGCTCCCTC GTGCGCTCTC CTGTTCCGAC CCTGCCGCTT 4680

ACCGGATACC TGTCCGCCTT TCTCCCTTCG GGAAGCGTGG CGCTTTCTCA ATGCTCACGC 4740

TGTAGGTATC TCAGTTCGGT GTAGGTCGTT CGCTCCAAGC TGGGCTGTGT GCACGAACCC 4800

CCCGTTCAGC CCGACCGCTG CGCCTTATCC GGTAACTATC GTCTTGAGTC CAACCCGGTA 4860

AGACACGACT TATCGCCACT GGCAGCAGCC ACTGGTAACA GGATTAGCAG AGCGAGGTAT 4920

GTAGGCGGTG CTACAGAGTT CTTGAAGTGG TGGCCTAACT ACGGCTACAC TAGAAGGACA 4980

GTATTTGGTA TCTGCGCTCT GCTGAAGCCA GTTACCTTCG GAAAAAGAGT TGGTAGCTCT 5040

TGATCCGGCA AACAAACCAC CGCTGGTAGC GGTGGTTTTT TTGTTTGCAA GCAGCAGATT 5100

ACGCGCAGAA AAAAAGGATC TCAAGAAGAT CCTTTGATCT TTTCTACGGG GTCTGACGCT 5160

CAGTGGAACG AAAACTCACG TTAAGGGATT TTGGTCATGA GATTATCAAA AAGGATCTTC 5220

ACCTAGATCC TTTTAAATTA AAAATGAAGT TTTAAATCAA TCTAAAGTAT ATATGAGTAA 5280

ACTTGGTCTG ACAGTTACCA ATGCTTAATC AGTGAGGCAC CTATCTCAGC GATCTGTCTA 5340

TTTCGTTCAT CCATAGTTGC CTGACTCCCC GTCGTGTAGA TAACTACGAT ACGGGAGGGC 5400

TTACCATCTG GCCCCAGTGC TGCAATGATA CCGCGAGACC CACGCTCACC GGCTCCAGAT 5460

TTATCAGCAA TAAACCAGCC AGCCGGAAGG GCCGAGCGCA GAAGTGGTCC TGCAACTTTA 5520

TCCGCCTCCA TCCAGTCTAT TAATTGTTGC CGGGAAGCTA GAGTAAGTAG TTCGCCAGTT 5580

AATAGTTTGC GCAACGTTGT TGCCATTGCT ACAGGCATCG TGGTGTCACG CTCGTCGTTT 5640

GGTATGGCTT CATTCAGCTC CGGTTCCCAA CGATCAAGGC GAGTTACATG ATCCCCCATG 5700

TTGTGCAAAA AAGCGGTTAG CTCCTTCGGT CCTCCGATCG TTGTCAGAAG TAAGTTGGCC 5760

GCAGTGTTAT CACTCATGGT TATGGCAGCA CTGCATAATT CTCTTACTGT CATGCCATCC 5820

GTAAGATGCT TTTCTGTGAC TGGTGAGTAC TCAACCAAGT CATTCTGAGA ATAGTGTATG 5880

CGGCGACCGA GTTGCTCTTG CCCGGCGTCA ATACGGGATA ATACCGCGCC ACATAGCAGA 5940

ACTTTAAAAG TGCTCATCAT TGGAAAACGT TCTTCGGGGC GAAAACTCTC AAGGATCTTA 6000

CCGCTGTTGA GATCCAGTTC GATGTAACCC ACTCGTGCAC CCAACTGATC TTCAGCATCT 6060

TTTACTTTCA CCAGCGTTTC TGGGTGAGCA AAAACAGGAA GGCAAAATGC CGCAAAAAAG 6120

GGAATAAGGG CGACACGGAA ATGTTGAATA CTCATACTCT TCCTTTTTCA ATATTATTGA 6180

AGCATTTATC AGGGTTATTG TCTCATGAGC GGATACATAT TTGAATGTAT TTAGAAAAAT 6240

AAACAAATAG GGGTTCCGCG CACATTTCCC CGAAAAGTGC CACCT 6285

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5703 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

GACGTCGCGG CCGCTCTAGG CCTCCAAAAA AGCCTCCTCA CTACTTCTGG AATAGCTCAG 60

AGGCCGAGGC GGCCTCGGCC TCTGCATAAA TAAAAAAAAT TAGTCAGCCA TGCATGGGGC 120

GGAGAATGGG CGGAACTGGG CGGAGTTAGG GGCGGGATGG GCGGAGTTAG GGGCGGGACT 180

ATGGTTGCTG ACTAATTGAG ATGCATGCTT TGCATACTTC TGCCTGCTGG GGAGCCTGGG 240

GACTTTCCAC ACCTGGTTGC TGACTAATTG AGATGCATGC TTTGCATACT TCTGCCTGCT 300

GGGGAGCCTG GGGACTTTCC ACACCCTAAC TGACACACAT TCCACAGAAT TAATTCCCGG 360

GGATCGATCC GTCGACGTAC GACTAGTTAT TAATAGTAAT CAATTACGGG GTCATTAGTT 420

CATAGCCCAT ATATGGAGTT CCGCGTTACA TAACTTACGG TAAATGGCCC GCCTGGCTGA 480

CCGCCCAACG ACCCCCGCCC ATTGACGTCA ATAATGACGT ATGTTCCCAT AGTAACGCCA 540

ATAGGGACTT TCCATTGACG TCAATGGGTG GACTATTTAC GGTAAACTGC CCACTTGGCA 600

GTACATCAAG TGTATCATAT GCCAAGTACG CCCCCTATTG ACGTCAATGA CGGTAAATGG 660

CCCGCCTGGC ATTATGCCCA GTACATGACC TTATGGGACT TTCCTACTTG GCAGTACATC 720

TACGTATTAG TCATCGCTAT TACCATGGTG ATGCGGTTTT GGCAGTACAT CAATGGGCGT 780

GGATAGCGGT TTGACTCACG GGGATTTCCA AGTCTCCACC CCATTGACGT CAATGGGAGT 840

TTGTTTTGGC ACCAAAATCA ACGGGACTTT CCAAAATGTC GTAACAACTC CGCCCCATTG 900

ACGCAAATGG GCGGTAGGCG TGTACGGTGG GAGGTCTATA TAAGCAGAGC TGGGTACGTG 960

AACCGTCAGA TCGCCTGGAG ACGCCATCGA ATTCATTGAT AGGATCCAGC AAGATGGTGT 1020

TGCAGACCCA GGTCTTCATT TCTCTGTTGC TCTGGATCTC TGGTGCCTAC GGGGATATCG 1080

TGATGACCCA GTCTCCAGAC TCGCTAGCTG TGTCTCTGGG CGAGAGGGCC ACCATCAACT 1140

GCAAGAGCTC TCAGAGTCTG TTAAACAGTG GAAATCAAAA GAACTACTTG GCCTGGTATC 1200

AGCAGAAACC CGGGCAGCCT CCTAAGTTGC TCATTTACGC GGCGTCGACT AGGGAATCTG 1260

GGGTACCTGA CCGATTCAGT GGCAGCGGGT CTGGGACAGA TTTCACTCTC ACCATCAGCA 1320

GCCTGCAGGC TGAAGATGTG GCAGTATACT ACTGTCAGAA TGTTCATAGT TTTCCATTCA 1380

CGTTCGGCGG AGGGACCAAG TTGGAGATCA AACGTACTGT GGCGGCGCCA TCTGTCTTCA 1440

TCTTCCCGCC ATCTGATGAG CAGTTGAAAT CTGGAACTGC CTCTGTTGTG TGCCTGCTGA 1500

ATAACTTCTA TCCCAGAGAG GCCAAAGTAC AGTGGAAGGT GGATAACGCC CTCCAATCGG 1560

GTAACTCCCA GGAGAGTGTC ACAGAGCAGG ACAGCAAGGA CAGCACCTAC AGCCTCAGCA 1620

GCACCCTGAC GCTGAGCAAA GCAGACTACG AGAAACACAA AGTCTACGCC TGCGAAGTCA 1680

CCCATCAGGG CCTGAGCTCG CCCGTCACAA AGAGCTTCAA CAGGGGAGAG TGTTAATTCT 1740

AGATCCGTTA TCTACGTATG ATCAGCCTCG ACTGTGCCTT CTAGTTGCCA GCCATCTGTT 1800

GTTTGCCCCT CCCCCGTGCC TTCCTTGACC CTGGAAGGTG CCACTCCCAC TGTCCTTTCC 1860

TAATAAAATG AGGAAATTGC ATCGCATTGT CTGAGTAGGT GTCATTCTAT TCTGGGGGGT 1920

GGGGTGGGGC AGGACAGCAA GGGGGAGGAT TGGGAAGACA ATAGCAGGCA TGCTGGGGAT 1980

GCGGTGGGCT CTATGGAACC AGCTGGGGCT CGACAGCTCG AGCTAGCTTT GCTTCTCAAT 2040

TTCTTATTTG CATAATGAGA AAAAAAGGAA AATTAATTTT AACACCAATT CAGTAGTTGA 2100

TTGAGCAAAT GCGTTGCCAA AAAGGATGCT TTAGAGACAG TGTTCTCTGC ACAGATAAGG 2160

ACAAACATTA TTCAGAGGGA GTACCCAGAG CTGAGACTCC TAAGCCAGTG AGTGGCACAG 2220

CATTCTAGGG AGAAATATGC TTGTCATCAC CGAAGCCTGA TTCCGTAGAG CCACACCTTG 2280

GTAAGGGCCA ATCTGCTCAC ACAGGATAGA GAGGGCAGGA GCCAGGGCAG AGCATATAAG 2340

GTGAGGTAGG ATCAGTTGCT CCTCACATTT GCTTCTGACA TAGTTGTGTT GGGAGCTTGG 2400

ATCGATCCAC CATGGTTGAA CAAGATGGAT TGCACGCAGG TTCTCCGGCC GCTTGGGTGG 2460

AGAGGCTATT CGGCTATGAC TGGGCACAAC AGACAATCGG CTGCTCTGAT GCCGCCGTGT 2520

TCCGGCTGTC AGCGCAGGGG CGCCCGGTTC TTTTTGTCAA GACCGACCTG TCCGGTGCCC 2580

TGAATGAACT GCAGGACGAG GCAGCGCGGC TATCGTGGCT GGCCACGACG GGCGTTCCTT 2640

GCGCAGCTGT GCTCGACGTT GTCACTGAAG CGGGAAGGGA CTGGCTGCTA TTGGGCGAAG 2700

TGCCGGGGCA GGATCTCCTG TCATCTCACC TTGCTCCTGC CGAGAAAGTA TCCATCATGG 2760

CTGATGCAAT GCGGCGGCTG CATACGCTTG ATCCGGCTAC CTGCCCATTC GACCACCAAG 2820

CGAAACATCG CATCGAGCGA GCACGTACTC GGATGGAAGC CGGTCTTGTC GATCAGGATG 2880

ATCTGGACGA AGAGCATCAG GGGCTCGCGC CAGCCGAACT GTTCGCCAGG CTCAAGGCGC 2940

GCATGCCCGA CGGCGAGGAT CTCGTCGTGA CCCATGGCGA TGCCTGCTTG CCGAATATCA 3000

TGGTGGAAAA TGGCCGCTTT TCTGGATTCA TCGACTGTGG CCGGCTGGGT GTGGCGGACC 3060

GCTATCAGGA CATAGCGTTG GCTACCCGTG ATATTGCTGA AGAGCTTGGC GGCGAATGGG 3120

CTGACCGCTT CCTCGTGCTT TACGGTATCG CCGCTCCCGA TTCGCAGCGC ATCGCCTTCT 3180

ATCGCCTTCT TGACGAGTTC TTCTGAGCGG GACTCTGGGG TTCGAAATGA CCGACCAAGC 3240

GACGCCCAAC CTGCCATCAC GAGATTTCGA TTCCACCGCC GCCTTCTATG AAAGGTTGGG 3300

CTTCGGAATC GTTTTCCGGG ACGCCGGCTG GATGATCCTC CAGCGCGGGG ATCTCATGCT 3360

GGAGTTCTTC GCCCACCCCA ACTTGTTTAT TGCAGCTTAT AATGGTTACA AATAAAGCAA 3420

TAGCATCACA AATTTCACAA ATAAAGCATT TTTTTCACTG CATTCTAGTT GTGGTTTGTC 3480

CAAACTCATC AATGTATCTT ATCATGTCTG GATCGCGGCC GCGATCCCGT CGAGAGCTTG 3540

GCGTAATCAT GGTCATAGCT GTTTCCTGTG TGAAATTGTT ATCCGCTCAC AATTCCACAC 3600

AACATACGAG CCGGAAGCAT AAAGTGTAAA GCCTGGGGTG CCTAATGAGT GAGCTAACTC 3660

ACATTAATTG CGTTGCGCTC ACTGCCCGCT TTCCAGTCGG GAAACCTGTC GTGCCAGCTG 3720

CATTAATGAA TCGGCCAACG CGCGGGGAGA GGCGGTTTGC GTATTGGGCG CTCTTCCGCT 3780

TCCTCGCTCA CTGACTCGCT GCGCTCGGTC GTTCGGCTGC GGCGAGCGGT ATCAGCTCAC 3840

TCAAAGGCGG TAATACGGTT ATCCACAGAA TCAGGGGATA ACGCAGGAAA GAACATGTGA 3900

GCAAAAGGCC AGCAAAAGGC CAGGAACCGT AAAAAGGCCG CGTTGCTGGC GTTTTTCCAT 3960

AGGCTCCGCC CCCCTGACGA GCATCACAAA AATCGACGCT CAAGTCAGAG GTGGCGAAAC 4020

CCGACAGGAC TATAAAGATA CCAGGCGTTT CCCCCTGGAA GCTCCCTCGT GCGCTCTCCT 4080

GTTCCGACCC TGCCGCTTAC CGGATACCTG TCCGCCTTTC TCCCTTCGGG AAGCGTGGCG 4140

CTTTCTCAAT GCTCACGCTG TAGGTATCTC AGTTCGGTGT AGGTCGTTCG CTCCAAGCTG 4200

GGCTGTGTGC ACGAACCCCC CGTTCAGCCC GACCGCTGCG CCTTATCCGG TAACTATCGT 4260

CTTGAGTCCA ACCCGGTAAG ACACGACTTA TCGCCACTGG CAGCAGCCAC TGGTAACAGG 4320

ATTAGCAGAG CGAGGTATGT AGGCGGTGCT ACAGAGTTCT TGAAGTGGTG GCCTAACTAC 4380

GGCTACACTA GAAGGACAGT ATTTGGTATC TGCGCTCTGC TGAAGCCAGT TACCTTCGGA 4440

AAAAGAGTTG GTAGCTCTTG ATCCGGCAAA CAAACCACCG CTGGTAGCGG TGGTTTTTTT 4500

GTTTGCAAGC AGCAGATTAC GCGCAGAAAA AAAGGATCTC AAGAAGATCC TTTGATCTTT 4560

TCTACGGGGT CTGACGCTCA GTGGAACGAA AACTCACGTT AAGGGATTTT GGTCATGAGA 4620

TTATCAAAAA GGATCTTCAC CTAGATCCTT TTAAATTAAA AATGAAGTTT TAAATCAATC 4680

TAAAGTATAT ATGAGTAAAC TTGGTCTGAC AGTTACCAAT GCTTAATCAG TGAGGCACCT 4740

ATCTCAGCGA TCTGTCTATT TCGTTCATCC ATAGTTGCCT GACTCCCCGT CGTGTAGATA 4800

ACTACGATAC GGGAGGGCTT ACCATCTGGC CCCAGTGCTG CAATGATACC GCGAGACCCA 4860

CGCTCACCGG CTCCAGATTT ATCAGCAATA AACCAGCCAG CCGGAAGGGC CGAGCGCAGA 4920

AGTGGTCCTG CAACTTTATC CGCCTCCATC CAGTCTATTA ATTGTTGCCG GGAAGCTAGA 4980

GTAAGTAGTT CGCCAGTTAA TAGTTTGCGC AACGTTGTTG CCATTGCTAC AGGCATCGTG 5040

GTGTCACGCT CGTCGTTTGG TATGGCTTCA TTCAGCTCCG GTTCCCAACG ATCAAGGCGA 5100

GTTACATGAT CCCCCATGTT GTGCAAAAAA GCGGTTAGCT CCTTCGGTCC TCCGATCGTT 5160

GTCAGAAGTA AGTTGGCCGC AGTGTTATCA CTCATGGTTA TGGCAGCACT GCATAATTCT 5220

CTTACTGTCA TGCCATCCGT AAGATGCTTT TCTGTGACTG GTGAGTACTC AACCAAGTCA 5280

TTCTGAGAAT AGTGTATGCG GCGACCGAGT TGCTCTTGCC CGGCGTCAAT ACGGGATAAT 5340

ACCGCGCCAC ATAGCAGAAC TTTAAAAGTG CTCATCATTG GAAAACGTTC TTCGGGGCGA 5400

AAACTCTCAA GGATCTTACC GCTGTTGAGA TCCAGTTCGA TGTAACCCAC TCGTGCACCC 5460

AACTGATCTT CAGCATCTTT TACTTTCACC AGCGTTTCTG GGTGAGCAAA AACAGGAAGG 5520

CAAAATGCCG CAAAAAAGGG AATAAGGGCG ACACGGAAAT GTTGAATACT CATACTCTTC 5580

CTTTTTCAAT ATTATTGAAG CATTTATCAG GGTTATTGTC TCATGAGCGG ATACATATTT 5640

GAATGTATTT AGAAAAATAA ACAAATAGGG GTTCCGCGCA CATTTCCCCG AAAAGTGCCA 5700

CCT 5703

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 81 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

ATCCAAAGAC AACTCCCGTA ACCAGGTTGT TCTGACCATG ACTAACATGG ACCCGGTTGA 60

CACCGCTACC TACTACTGCG C 81

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 85 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

TCGAGCGCAG TAGTAGGTAG CGGTGTCAAC CGGGTCCATG TTAGTCATGG TCAGAACAAC 60

CTGGTTACGG GAGTTGTCTT TGGAT 85

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 73 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

AACCTGCACC GTCTCCGGTT TCTCCCTGAC GAGCTATAGT GTACACTGGA TCCGTCAGCC 60

GCCGGGTAAA GGT 73

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 77 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

CTAGACCTTT ACCCGGCGGC TGACGGATCC AGTGTACACT ATAGCTCGTC AGGGAGAAAC 60

CGGAGACGGT GCAGGTT 77

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 46 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

CTAGCTGTGT CAGCTGGCGA GAGGGCCACC ATCAACTGCA AGAGCT 46

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

CTTGCAGTTG ATGGTGGCCC TCTCGCCAGC TGACACAG 38

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 140 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

TTCGAGGACG CCAGCAACAT GGTGTTGCAG ACCCAGGTCT TCATTTCTCT GTTGCTCTGG 60

ATCTCTGGTG CCTACGGGCA GGTCCAACTG CAGGAGAGCG GTCCAGGTCT TGTGAGACCT 120

AGCCAGACCC TGAGCCTGAC 140

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 138 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

GTGCCTCCAC TAGCCCATAT TACTCCAAGC CACTCTAGAC CTCGTCCAGG TGGCTGTCTC 60

ACCCAGTGTA CACTATAGCT GGTGAGGGAG AAGCCCGAGA CGGTGCAGGT CAGGCTCAGG 120

GTCTGGCTAG GTCTCACA 138

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 143 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

GGCTTGGAGT AATATGGGCT AGTGGAGGCA CAGATTATAA TTCGGCTCTC ATGTCCAGAC 60

TGAGTATACT GAAAGACAAC AGCAAGAACC AGGTCAGCCT GAGACTCAGC AGCGTGACAG 120

CCGCCGACAC CGCGGTCTAT TTC 143

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 136 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

CCAGTGCCAA GCTTGGGCCC TTGGTGGAGG CGCTCGAGAC GGTGACCGTG GTACCTTGTC 60

CCCAGTAGTC AAGCCGTAGT AAGGAAGAAG GGGGATCTCG AGCACAGAAA TAGACCGCGG 120

TGTCGGCGGC TGTCAC 136

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 357 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

CAGGTCCAAC TGCAGGAGAG CGGTCCAGGT CTTGTGAGAC CTAGCCAGAC CCTGAGCCTG 60

ACCTGCACCG TCTCGGGCTT CTCCCTCACC AGCTATAGTG TACACTGGGT GAGACAGCCA 120

CCTGGACGAG GTCTAGAGTG GCTTGGAGTA ATATGGGCTA GTGGAGGCAC AGATTATAAT 180

TCGGCTCTCA TGTCCAGACT GAGTATACTG AAAGACAACA GCAAGAACCA GGTCAGCCTG 240

AGACTCAGCA GCGTGACAGC CGCCGACACC GCGGTCTATT ACTGTGCTCG GGATCCCCCT 300

TCTTCCTTAC TACGGCTTGA CTACTGGGGA CAAGGTACCA CGGTCACCGT CTCGAGC 357

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 119 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Ser Tyr
20 25 30

Ser Val His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Leu
35 40 45

Gly Val Ile Trp Ala Ser Gly Gly Thr Asp Tyr Asn Ser Ala Leu Met
50 55 60

Ser Arg Leu Ser Ile Leu Lys Asp Asn Ser Lys Asn Gln Val Ser Leu
65 70 75 80

Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg Asp Pro Pro Ser Ser Leu Leu Arg Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Thr Val Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

AGGACGCCAG CAACATGGTG TTGCAGAC 28

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

TGCCAAGCTT GGGCCCTTGG TGGAGGCGCT CGAGAC 36

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 121 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

GACCATGATT ACGAATTCGT AGTCGGATAT CGTGATGACC CAGAGCCCAA GCAGCCTGAG 60

CGCTAGCGTG GGTGACAGAG TGACCATCAC CTGTAAGAGC TCTCAGAGTC TGTTAAACAG 120

T 121

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 116 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

AGATTCCCTA GTCGATGCCC CGTAGATCAG CAGCTTTGGA GCCTTACCGG GTTTCTGCTG 60

ATACCAGGCC AAGTAGTTCT TTTGATTTCC ACTGTTTAAC AGACTCTGAG AGCTCT 116

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 116 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

TCTACGGGGC ATCGACTAGG GAATCTGGGG TACCAGATAG ATTCAGCGGT AGCGGTAGCG 60

GAACCGACTT CACCTTCACC ATCAGCAGCC TGCAGCCAGA GGACATCGCC ACCTAC 116

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 117 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

TCGATGCCAA GCTTGGCGCC GCCACAGTAC GTTTGATCTC CACCTTGGTC CCTTGTCCGA 60

ACGTGAATGG AAAACTATGA ACATTCTGGC AGTAGTAGGT GGCGATGTCC TCTGGCT 117

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 339 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

GATATCGTGA TGACCCAGAG CCCAAGCAGC CTGAGCGCTA GCGTGGGTGA CAGAGTGACC 60

ATCACCTGTA AGAGCTCTCA GAGTCTGTTA AACAGTGGAA ATCAAAAGAA CTACTTGGCC 120

TGGTATCAGC AGAAACCCGG TAAGGCTCCA AAGCTGCTGA TCTACGGGGC ATCGACTAGG 180

GAATCTGGGG TACCAGATAG ATTCAGCGGT AGCGGTAGCG GAACCGACTT CACCTTCACC 240

ATCAGCAGCC TGCAGCCAGA GGACATCGCC ACCTACTACT GCCAGAATGT TCATAGTTTT 300

CCATTCACGT TCGGACAAGG GACCAAGGTG GAGATCAAA 339

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 113 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

Asp Ile Val Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser
20 25 30

Gly Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys
35 40 45

Ala Pro Lys Leu Leu Ile Tyr Gly Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr
65 70 75 80

Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Asn
85 90 95

Val His Ser Phe Pro Phe Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
100 105 110

Lys

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

GATTACGAAT TCGTAGTCGG ATAT 24

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

TGCCAAGCTT GGCGCCGCCA CAGT 24

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

CTAGTGCGGG TGACCGAGTG ACCATCACCT GTAAGAGCT 39

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 31 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

CTTACAGGTG ATGGTCACTC GGTCACCCGC A 31

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 66 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

GGTCTATTAC TGTGCTCGGG ATCCCCCTTC TTCCTTACTA CGGCTTGACT ACTGGGGACA 60

AGGTAC 66

(2) INFORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 64 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

CTTGTCCCCA GTAGTCAAGC CGTAGTAAGG AAGAAGGGGG ATCCCGAGCA CAGTAATAGA 60

CCGC 64

WHAT IS CLAIMED IS:

1. An improved method for treating conditions associated with excess eosinophil production wherein the improvement comprises the step of administering a neutralizing monoclonal antibody for human IL-5 which does not block binding of human IL-5 to the α-chain of the human IL-5 receptor.

2. The method according to claim 1 wherein the monoclonal antibody has the identifying characteristics of mAb 4A6.

3. The method according to claim 1 wherein the monoclonal antibody is an altered antibody comprising a heavy chain and a light chain, wherein the framework regions of said heavy and light chains are derived from at least one selected antibody and the amino acid sequences of the complementarity determining regions of each said chain are derived from the monoclonal antibody 4A6.

4. The method of claim 1 wherein said condition associated with excess eosinophil production is asthma.

5. The method of claim 1 wherein said condition associated with excess eosinophil production is allergic rhinitis.

6. The method of claim 1 wherein said condition associated with excess eosinophil production is atopic dermatitis.

7. A method to assess the presence or absence of a human IL-5 soluble receptor α-chain/human IL-5 complex in a human which comprises obtaining a sample of biological fluid from a patient and allowing a monoclonal antibody for human IL-5 which does not block binding of human IL-5 to the α-chain of the human IL-5 receptor to come in contact with such sample under conditions such that a human IL-5 soluble receptor α-chain/human IL-5/monoclonal antibody complex can form and detecting the presence or absence of said human IL-5 soluble receptor α-chain/human IL-5/ monoclonal antibody complex.

8. A method for aiding in the diagnosis of allergies and other conditions associated with excess eosinophil production comprising the steps of determining the amount of human IL-5 soluble receptor α-chain/human IL-5 complex in a

sample of a patient according to the method of claim 7 and comparing that to the mean amount of human IL-5 soluble receptor α-chain/human IL-5 complex in the normal population, whereby the presence of significantly elevated amount of human IL-5 soluble receptor α-chain/human IL-5 complex in the patient is an indication of allergies and other conditions associated with excess eosinophil production.

9. A method of screening compounds to identify those compounds which antagonize binding of a human IL-5 soluble receptor α-chain/human IL-5 complex to a human IL-5 receptor β-chain which method comprises contacting the human IL-5 receptor β-chain with a plurality of candidate compounds under conditions to permit binding to the receptor and identifying those candidate compounds that antagonize binding of a human IL-5 soluble receptor α-chain/human IL-5 complex.

10. A method of screening compounds to identify those compounds which antagonize binding of the following complex to a human IL-5 receptor β-chain:

a human IL-5 soluble receptor α-chain / human IL-5 / monoclonal antibody for human IL-5 which does not block binding of human IL-5 to the α-chain of the human IL-5 receptor;

which method comprises contacting the human IL-5 receptor β-chain with a plurality of candidate compounds under conditions to permit binding to the IL-5 receptor β-chain and identifying those candidate compounds that antagonize binding of said receptor/IL-5/antibody complex to the IL-5 receptor β-chain.

FIGURE 1

2B6 Heavy Chain Variable Region DNA Sequence.

```
1                                                   CCT GGC    30
                                                    Pro Gly

31  CTG GTG GCG CCC TCA CAG AGC CTG TCC ATC         60
    Leu Val Ala Pro Ser Gln Ser Leu Ser Ile

61  ACT TGC ACT GTC TCT GGG TTT TCA TTA ACC         90
    Thr Cys Thr Val Ser Gly Phe Ser Leu Thr

91  AGC TAT AGT GTA CAC TGG GTT CGC CAG CCT         120
    Ser Tyr Ser Val His Trp Val Arg Gln Pro

121 CCA GGA AAG GGT CTG GAG TGG CTG GGA GTA         150
    Pro Gly Lys Gly Leu Glu Trp Leu Gly Val

151 ATA TGG GCT AGT GGA GGC ACA GAT TAT AAT         180
    Ile Trp Ala Ser Gly Gly Thr Asp Tyr Asn

181 TCG GCT CTC ATG TCC AGA CTG AGC ATC AGC         210
    Ser Ala Leu Met Ser Arg Leu Ser Ile Ser

211 AAA GAC AAC TCC AAG AGC CAA GTT TTC TTA         240
    Lys Asp Asn Ser Lys Ser Gln Val Phe Leu

241 AAA CTG AAC AGT CTG CAA ACT GAT GAC ACA         270
    Lys Leu Asn Ser Leu Gln Thr Asp Asp Thr

271 GCC ATG TAC TAC TGT GCC AGA GAT CCC CCT         300
    Ala Met Tyr Tyr Cys Ala Arg Asp Pro Pro

301 TCT TCC TTA CTA CGG CTT GAC TAC TGG GGC         330
    Ser Ser Leu Leu Arg Leu Asp Tyr Trp Gly

331 CAA GGC ACC ACT CTC ACA GTC TCC TCA     357
    Gln Gly Thr Thr Leu Thr Val Ser Ser
```

FIGURE 2

2B6 Light Chain Variable Region DNA Sequence

```
1                                                   TCC TCC    30
  Asp Ile Val Met Thr Gln Ser Pro Ser Ser

31  CTG AGT GTG TCA GCA GGA GAG AAG GTC ACT    60
  Leu Ser Val Ser Ala Gly Glu Lys Val Thr

61  ATG AGC TGC AAG TCC AGT CAG AGT CTG TTA    90
  Met Ser Cys Lys Ser Ser Gln Ser Leu Leu

91  AAC AGT GGA AAT CAA AAG AAC TAC TTG GCC   120
  Asn Ser Gly Asn Gln Lys Asn Tyr Leu Ala

121 TGG TAC CAG CAG AAA CCA GGG CAG CCT CCT   150
  Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro

151 AAA CTT TTG ATC TAC GGG GCA TCC ACT AGG   180
  Lys Leu Leu Ile Tyr Gly Ala Ser Thr Arg

181 GAA TCT GGG GTC CCT GAT CGC TTC ACA GGC   210
  Glu Ser Gly Val Pro Asp Arg Phe Thr Gly

211 AGT GGA TCT GGA ACC GAT TTC ACT CTT TCC   240
  Ser Gly Ser Gly Thr Asp Phe Thr Leu Ser

241 ATC AGC AGT GTG CAG GCT GAA GAC CTG GCA   270
  Ile Ser Ser Val Gln Ala Glu Asp Leu Ala

271 GTT TAT TAC TGT CAG AAT GTT CAT AGT TTT   300
  Val Tyr Tyr Cys Gln Asn Val His Ser Phe

301 CCA TTC ACG TTC GGC TCG GGG ACA GAG TTG   330
  Pro Phe Thr Phe Gly Ser Gly Thr Glu Leu

331 GAA ATA AAA    339
  Glu Ile Lys
```

FIGURE 3

2F2 Heavy Chain Variable Region DNA Sequence

```
1    CCT GGC CTG GTG GCG CCC TCA CAG AGC CTG    30
     Pro Gly Leu Val Ala Pro Ser Gln Ser Leu

31   TCC ATC ACT TGC ACT GTC TCT GGG TTT TCA    60
     Ser Ile Thr Cys Thr Val Ser Gly Phe Ser

61   TTA ACC AGT TAT AGT GTA CAC TGG GTT CGC    90
     Leu Thr Ser Tyr Ser Val His Trp Val Arg

91   CAG CCT CCA GGA AAG GGT CTG GAG TGG CTG   120
     Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu

121  GGA GTA ATA TGG GCT AGT GGA GGC ACA GAT   150
     Gly Val Ile Trp Ala Ser Gly Gly Thr Asp

151  TAT AAt TCG GCT CTC ATG TCC AGA CTG AGC   180
     Tyr Asn Ser Ala Leu Met Ser Arg Leu Ser

181  ATC AGC AAA GAC AAC TCC AAG AGC CAA GTT   210
     Ile Ser Lys Asp Asn Ser Lys Ser Gln Val

211  TTC TTA AAA CTG AAC AGT CTG CGA ACT GAT   240
     Phe Leu Lys Leu Asn Ser Leu Arg Thr Asp

241  GAC ACA GCC ATG TAC TAC TGT GCC AGA GAT   270
     Asp Thr Ala Met Tyr Tyr Cys Ala Arg Asp

271  CCC CCT TCT TCC TTA CTA CGG CTT GAC TAC   300
     Pro Pro Ser Ser Leu Leu Arg Leu Asp Tyr

301  TGG GGC CAA GGC ACC ACT CTC ACA GTC TCC   330
     Trp Gly Gln Gly Thr Thr Leu Thr Val Ser

331  TCA    333
     Ser
```

FIGURE 4

2F2 Light Chain Variable Region DNA Sequence

```
     TCC TCC CTG AGT GTG TCA GCA GGA GAG AAG     30
     Ser Ser Leu Ser Val Ser Ala Gly Glu Lys

31   GTC ACT ATG AGC TGC AAG TCC AGT CAG AGT     60
     Val Thr Met Ser Cys Lys Ser Ser Gln Ser

61   CTA TTA AAC AGT GGA AAT CAA AAG AAC TAC     90
     Leu Leu Asn Ser Gly Asn Gln Lys Asn Tyr

91   TTG GCC TGG TAC CAA CAG AAA CCA GGG CAG    120
     Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln

121  CCT CCT AAA CTT TTG ATC TAC GGG GCA TCC    150
     Pro Pro Lys Leu Leu Ile Tyr Gly Ala Ser

151  ACT AGG GAA TCT GGG GTC CCT GAT CGC TTC    180
     Thr Arg Glu Ser Gly Val Pro Asp Arg Phe

181  ACA GGC AGT GGA TCT GGA ACC GAT TTC ACT    210
     Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr

211  CTT ACC ATC AGC AGT GTG CAG GCT GAA GAC    240
     Leu Thr Ile Ser Ser Val Gln Ala Glu Asp

241  CTG GCA GTT TAT TAC TGT CAG AAT GAT CAT    270
     Leu Ala Val Tyr Tyr Cys Gln Asn Asp His

271  AGT TTT CCA TTC ACG TTC GGC TCG GGG ACA    300
     Ser Phe Pro Phe Thr Phe Gly Ser Gly Thr

301  GAG TTG GAA ATA AAA    315
     Glu Leu Glu Ile Lys
```

FIGURE 5

2E3 Heavy Chain Variable Region DNA Sequence

```
   1  CCT GGC CTG GTG GCG CCC TCA CAG AGC CTG   30
      Pro Gly Leu Val Ala Pro Ser Gln Ser Leu

  31  TCC ATC ACT TGC ACT GTC TCT GGG TTT TCA   60
      Ser Ile Thr Cys Thr Val Ser Gly Phe Ser

  61  TTA ACC AGC TAT AGT GTA CAC TGG GTT CGC   90
      Leu Thr Ser Tyr Ser Val His Trp Val Arg

  91  CAG CCT CCA GGA AAG GGT CTG GAG TGG CTG  120
      Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu

 121  GGA GTA ATC TGG GCT AGT GGA GGC ACA GAT  150
      Gly Val Ile Trp Ala Ser Gly Gly Thr Asp

 151  TAT AAT TCG GCT CTC ATG TCC AGA CTG AGC  180
      Tyr Asn Ser Ala Leu Met Ser Arg Leu Ser

 181  ATC AGC AAA GAC AAC TCC AAG AGC CAA GTT  210
      Ile Ser Lys Asp Asn Ser Lys Ser Gln Val

 211  TTC TTA AAA CTG AAC AGT CTG CAA ACT GAT  240
      Phe Leu Lys Leu Asn Ser Leu Gln Thr Asp

 241  GAC GCA GCC ATG TAC TAC TGT GCC AGA GAT  270
      Asp Ala Ala Met Tyr Tyr Cys Ala Arg Asp

 271  CCC CCT TTT TCC TTA CTA CGG CTT GAC TTC  300
      Pro Pro Phe Ser Leu Leu Arg Leu Asp Phe

 301  TGG GGC CAA GGC ACC ACT CTC ACA GTC TCC  330
      Trp Gly Gln Gly Thr Thr Leu Thr Val Ser

 331  TCA  333
      Ser
```

FIGURE 6

2E3 Light Chain Variable Region DNA Sequence

```
1    TCC TCT CTG AGT GTG TCA GCA GGA GAG AAG    30
     Ser Ser Leu Ser Val Ser Ala Gly Glu Lys

31   GTC ACT ATG AGC TGC AAG TCC AGT CAG AGT    60
     Val Thr Met Ser Cys Lys Ser Ser Gln Ser

61   CTG TTA AAC AGT GGA AAT CAA AAA AAC TAC    90
     Leu Leu Asn Ser Gly Asn Gln Lys Asn Tyr

91   TTG GCC TGG TAC CAG CAG AAA CCA GGG CAG   120
     Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln

121  CCT CCT AAA CTT TTG ATC TAC GGG GCA TCC   150
     Pro Pro Lys Leu Leu Ile Tyr Gly Ala Ser

151  ACT AGG GAA TCT GGG GTC CCT GAT CGC TTC   180
     Thr Arg Glu Ser Gly Val Pro Asp Arg Phe

181  ACA GGC AGT GGA TCT GGA ACC GAT TTC ACT   210
     Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr

211  CTT ACC ATC AGC AGT GTG CAG GCT GAA GAC   240
     Leu Thr Ile Ser Ser Val Gln Ala Glu Asp

241  CTG GCA GTT TAT TAC TGT CAG AAT GAT CAT   270
     Leu Ala Val Tyr Tyr Cys Gln Asn Asp His

271  AGT TTT CCA TTC ACG TTC GGC TCG GGG ACA   300
     Ser Phe Pro Phe Thr Phe Gly Ser Gly Thr

301  GAG TTG GAA ATA AAA   315
     Glu Leu Glu Ile Lys
```

FIGURE 7

2B6 CDRs

Heavy Chain 1: SYSVH
Heavy Chain 2: VIWASGGTDYNSALMS
Heavy Chain 3: DPPSSLLRLDY

Light Chain 1: KSSQSLLNSGNQKNYLA
Light Chain 2: GASTRES
Light Chain 3: QNVHSFPFT

2F2 CDRs

Heavy Chain 1: SYSVH
Heavy Chain 2: VIWASGGTDYNSALMS
Heavy Chain 3: DPPSSLLRLDY

Light Chain 1: KSSQSLLNSGNQKNYLA
Light Chain 2: GASTRES
Light Chain 3: QNDHSFPFT

2E3 CDRs

Heavy Chain 1: SYSVH
Heavy Chain 2: VIWASGGTDYNSALMS
Heavy Chain 3: DPPFSLLRLDF

Light Chain 1: KSSQSLLNSGNQKNYLA
Light Chain 2: GASTRES
Light Chain 3: QNDHSFPFT

FIGURE 8

ILS Humanized Heavy Chain Variable Region:

```
  1  CAG GTT ACC CTG CGT GAA TCC GGT CCG GCA   30
     Gln Val Thr Leu Arg Glu Ser Gly Pro Ala

 31  CTA GTT AAA CCG ACC CAG ACC CTG ACG TTA   60
     Leu Val Lys Pro Thr Gln Thr Leu Thr Leu

 61  ACC TGC ACC TTC TCC GGT TTC TCC CTG ACG   90
     Thr Cys Thr Phe Ser Gly Phe Ser Leu Thr

 91  AGC TAT AGT GTA CAC TGG ATC CGT CAG CCG   120
     Ser Tyr Ser Val His Trp Ile Arg Gln Pro

121  CCG GGT AAA GGT CTG GAG TGG CTG GGT GTA   150
     Pro Gly Lys Gly Leu Glu Trp Leu Gly Val

151  ATA TGG GCT AGT GGA GGC ACA GAT TAT AAT   180
     Ile Trp Ala Ser Gly Gly Thr Asp Tyr Asn

181  TCG GCT CTC ATG TCC CGT CTG ACG ATA TCC   210
     Ser Ala Leu Met Ser Arg Leu Thr Ile Ser

211  AAA GAC ACC TCC CGT AAC CAG GTT GTT CTG   240
     Lys Asp Thr Ser Arg Asn Gln Val Val Leu

241  ACC ATG ACT AAC ATG GAC CCG GTT GAC ACC   270
     Thr Met Thr Asn Met Asp Pro Val Asp Thr

271  GCT ACC TAC TAC TGC GCT CGA GAT CCC CCT   300
     Ala Thr Tyr Tyr Cys Ala Arg Asp Pro Pro

301  TCT TCC TTA CTA CGG CTT GAC TAC TGG GGT   330
     Ser Ser Leu Leu Arg Leu Asp Tyr Trp Gly

331  CGT GGT ACC CCA GTT ACC GTG AGC TCA   357
     Arg Gly Thr Pro Val Thr Val Ser Ser
```

FIGURE 9

Humanized Light Chain Variable Region

```
1   GAT ATC GTG ATG ACC CAG TCT CCA GAC TCG   30
    Asp Ile Val Met Thr Gln Ser Pro Asp Ser

31  CTA GCT GTG TCT CTG GGC GAG AGG GCC ACC   60
    Leu Ala Val Ser Leu Gly Glu Arg Ala Thr

61  ATC AAC TGC AAG AGC TCT CAG AGT CTG TTA   90
    Ile Asn Cys Lys Ser Ser Gln Ser Leu Leu

91  AAC AGT GGA AAT CAA AAG AAC TAC TTG GCC   120
    Asn Ser Gly Asn Gln Lys Asn Tyr Leu Ala

121 TGG TAT CAG CAG AAA CCC GGG CAG CCT CCT   150
    Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro

151 AAG TTG CTC ATT TAC GGG GCG TCG ACT AGG   180
    Lys Leu Leu Ile Tyr Gly Ala Ser Thr Arg

181 GAA TCT GGG GTA CCT GAC CGA TTC AGT GGC   210
    Glu Ser Gly Val Pro Asp Arg Phe Ser Gly

211 AGC GGG TCT GGG ACA GAT TTC ACT CTC ACC   240
    Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr

241 ATC AGC AGC CTG CAG GCT GAA GAT GTG GCA   270
    Ile Ser Ser Leu Gln Ala Glu Asp Val Ala

271 GTA TAC TAC TGT CAG AAT GTT CAT AGT TTT   300
    Val Tyr Tyr Cys Gln Asn Val His Ser Phe

301 CCA TTC ACG TTC GGC GGA GGG ACC AAG TTG   330
    Pro Phe Thr Phe Gly Gly Gly Thr Lys Leu

331 GAG ATC AAA   339
    Glu Ile Lys
```

Figure 10

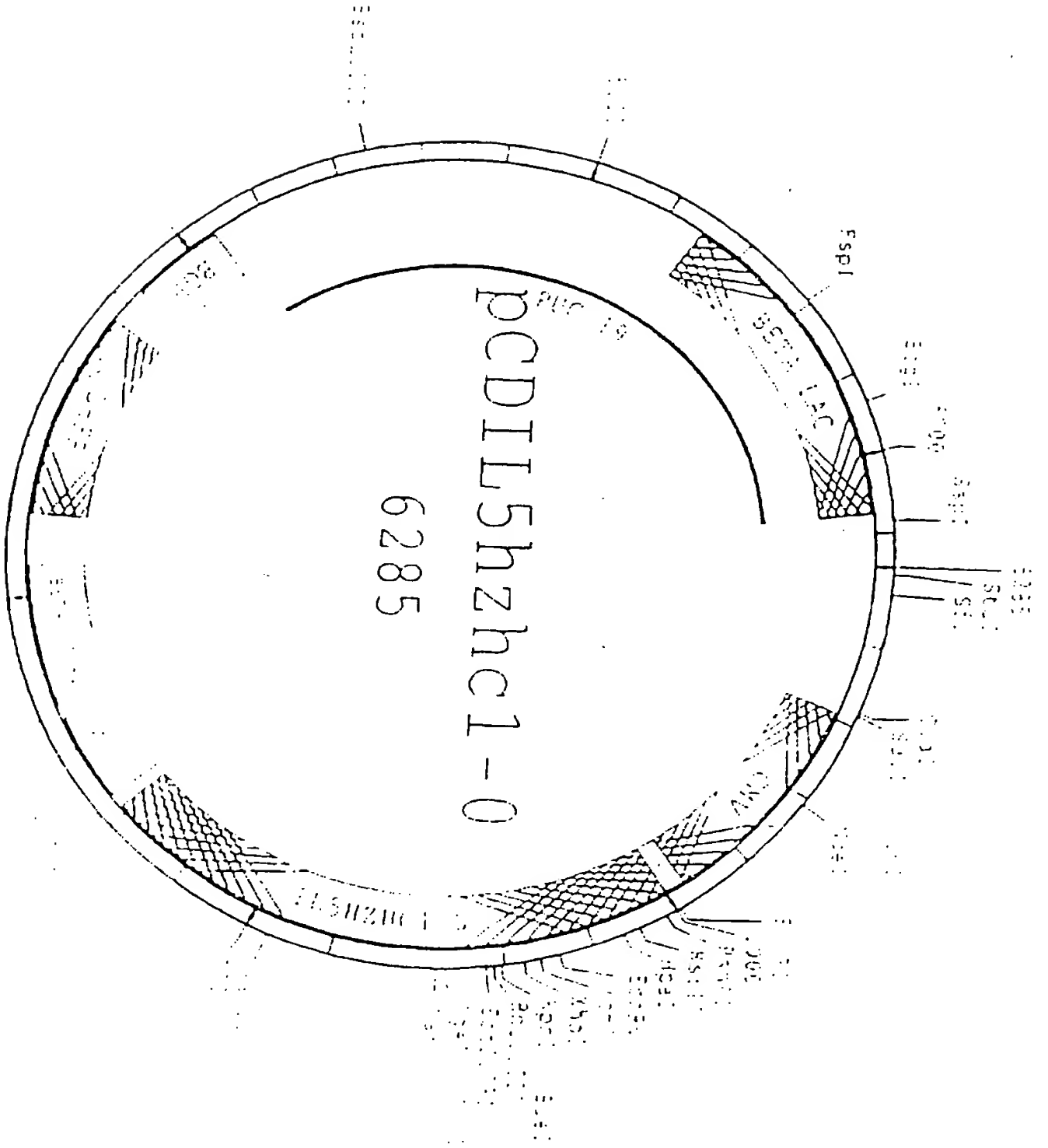

FIGURE 11

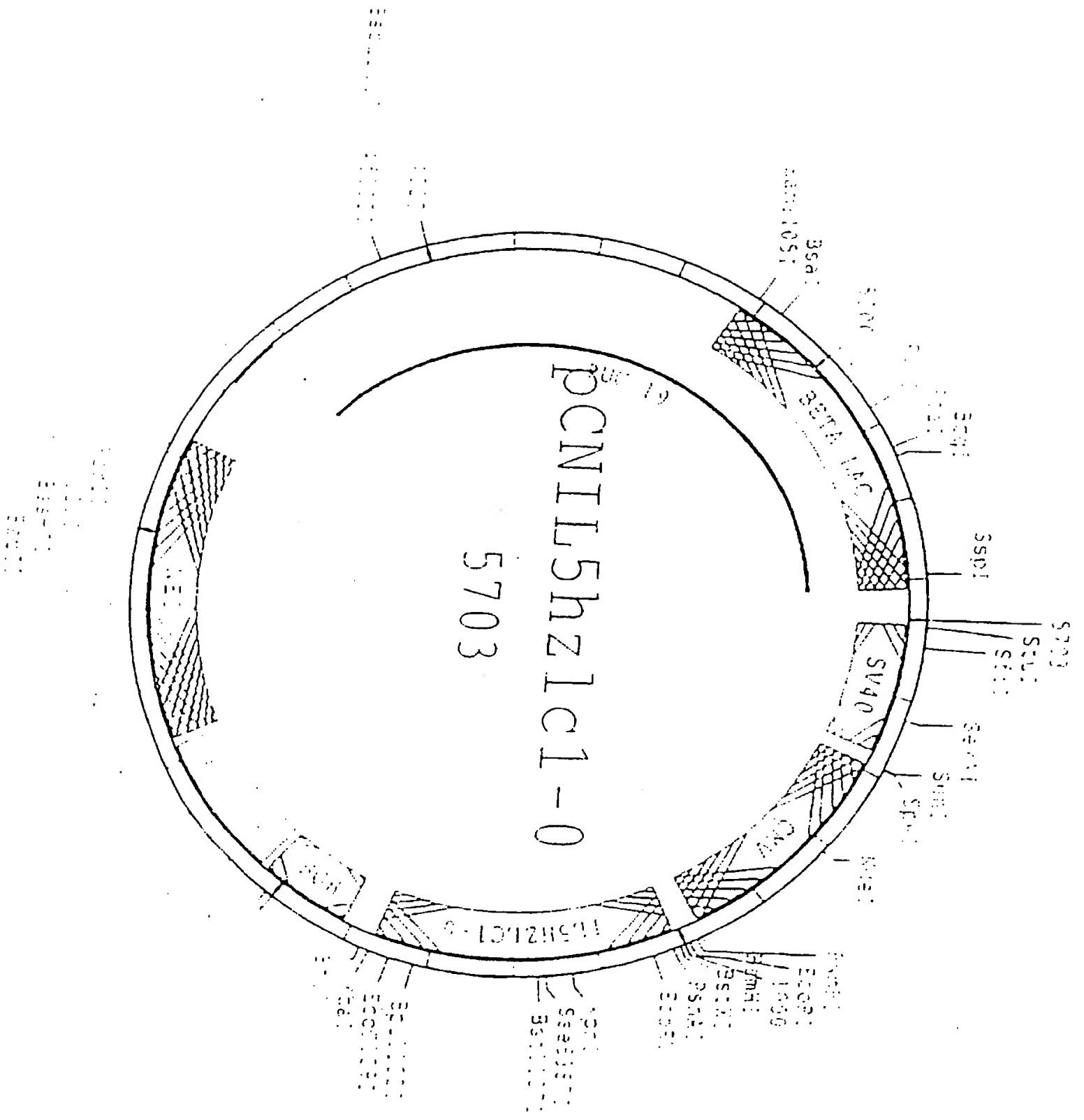

FIGURE 12

[illegible] Humanized Heavy Chain Variable Region

```
  1  CTG GTC CAA CTG CAG GAG AGC GGT CCA GGT   30
     Gln Val Gln Leu Gln Glu Ser Gly Pro Gly

 31  CTT GTG AGA CCT AGC CAG ACC CTG AGC CTG   60
     Leu Val Arg Pro Ser Gln Thr Leu Ser Leu

 61  ACC TGC ACC GTC TCG GGC TTC TCC CTC ACC   90
     Thr Cys Thr Val Ser Gly Phe Ser Leu Thr

 91  AGC TAT AGT GTA CAC TGG GTG AGA CAG CCA  120
     Ser Tyr Ser Val His Trp Val Arg Gln Pro

121  CCT GGA CGA GGT CTA GAG TGG CTT GGA GTA  150
     Pro Gly Arg Gly Leu Glu Trp Leu Gly Val

151  ATA TGG GCT AGT GGA GGC ACA GAT TAT AAT  180
     Ile Trp Ala Ser Gly Gly Thr Asp Tyr Asn

181  TCG GCT CTC ATG TCC AGA CTG TCA ATA CTG  210
     Ser Ala Leu Met Ser Arg Leu Ser Ile Leu

211  AAA GAC AAC AGC AAG AAC CAG GTC AGC CTG  240
     Lys Asp Asn Ser Lys Asn Gln Val Ser Leu

241  AGA CTC AGC AGC GTG ACA GCC GCC GAC ACC  270
     Arg Leu Ser Ser Val Thr Ala Ala Asp Thr

271  GCG GTC TAT TAC TGT GCT CGG GAT CCC CCT  300
     Ala Val Tyr Tyr Cys Ala Arg Asp Pro Pro

301  TCT TCC TTA CTA CGG CTT GAC TAC TGG GGA  330
     Ser Ser Leu Leu Arg Leu Asp Tyr Trp Gly

331  CAA GGT ACC ACG GTC ACC GTC TCG AGC   357
     Gln Gly Thr Thr Val Thr Val Ser Ser
```

FIGURE 13

11C6.RE1 Humanized Light Chain Variable Region

```
   1  GAT ATC GTG ATG ACC CAG AGC CCA AGC AGC    30
      Asp Ile Val Met Thr Gln Ser Pro Ser Ser

  31  CTG AGC GCT AGC GTG GGT GAC AGA GTG ACC    60
      Leu Ser Ala Ser Val Gly Asp Arg Val Thr

  61  ATC ACC TGT AAG AGC TCT CAG AGT CTG TTA    90
      Ile Thr Cys Lys Ser Ser Gln Ser Leu Leu

  91  AAC AGT GGA AAT CAA AAG AAC TAC TTG GCC   120
      Asn Ser Gly Asn Gln Lys Asn Tyr Leu Ala

 121  TGG TAT CAG CAG AAA CCC GGT AAG GCT CCA   150
      Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro

 151  AAG CTG CTG ATC TAC GGG GCA TCG ACT AGG   180
      Lys Leu Leu Ile Tyr Gly Ala Ser Thr Arg

 181  GAA TCT GGG GTA CCA GAT AGA TTC AGC GGT   210
      Glu Ser Gly Val Pro Asp Arg Phe Ser Gly

 211  AGC GGT AGC GGA ACC GAC TTC ACC TTC ACC   240
      Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr

 241  ATC AGC AGC CTG CAG CCA GAG GAC ATC GCC   270
      Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala

 271  ACC TAC TAC TGC CAG AAT GTT CAT AGT TTT   300
      Thr Tyr Tyr Cys Gln Asn Val His Ser Phe

 301  CCA TTC ACG TTC GGA CAA GGG ACC AAG GTG   330
      Pro Phe Thr Phe Gly Gln Gly Thr Lys Val

 331  GAG ATC AAA   339
      Glu Ile Lys
```

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/10769

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 39/395, 39/40; G01N 33/53, 33/567
US CL :424/130.1, 133.1, 137.1, 141.1, 145.1; 435/7.1, 7.2

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/130.1, 133.1, 137.1, 141.1, 145.1; 435/7.1, 7.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, BIOSIS, CA, EMBASE, WPIDS
terms: human interleukin-5, alpha chain, antibody, eosinophilia

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	EP 0 367 596 A1 (SCHERING CORPORATION) 09 May 1990 (09.05.90), see entire document.	1 --- 2-10
X	MCNAMEE et al. Production, characterisation and use of monoclonal antibodies to human interleukin-5 in an enzyme-linked immunosorbent assay. Journal of Immunological Methods. August 1991, Vol. 141, No. 1, pages 81-88, see entire document.	1
Y	MORRISON et al. Chimeric human antibody molecules: Mouse antigen-binding domains with human constant region domains. Proc. Natl. Acad. Sci. USA. November 1984, Vol. 81, pages 6851-6855, see entire document.	2-6

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 04 AUGUST 1997	Date of mailing of the international search report 29 AUG 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer MARK NAVARRO Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/10769

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,980,359 A (HASSPACHER et al) 25 December 1990 (25/12/90), see entire document.	2-6
Y	US 5,455,337 A (DEVOS et al) 03 October 1995 (03/10/95), see entire document.	7-10